Biocatalysis in organic synthesis

Aoife M. Foley, B.Sc.

A thesis presented for the degree of

Doctor of Philosophy

to

The National University of Ireland

School of Chemistry

University College Cork

Supervisor: Prof. Anita R. Maguire

Head of Department: Prof. Justin D. Holmes

January 2019

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

_____

Aoife M. Foley

January 2019

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Prof. Anita Maguire for her support, guidance and encouragement throughout the course of this research and especially while writing this thesis. I am very grateful for the opportunities she has afforded me. Sincere thanks are due to Dr. Dan McCarthy, Dr. Lorraine Bateman and Dr. Denis Lynch for NMR services; Dr. Florence McCarthy and Mick O'Shea for mass spectrometry services; Barry O'Mahony and Noel Browne for microanalysis; Dr. Simon Lawrence, Dr. Udaya Khandavilli, and Dr. Lorraine Crowley for crystallography services; Derry Kearny for glass blowing services, and to the technical staff Tina Kent, Denis Duggan, Johnny Ryan, Dr. Mattias Jauch, Dr. Trevor Carey, Dr. Ian O'Connor (for fixing the printer on more than one occasion), and Dr. Tom O'Mahony. Dr. Nuala Maguire for help and guidance with HPLC analysis, getting started, getting finished, and all of the troubleshooting in between. Special thanks to Debbie Curran, for pulling meetings out of nowhere and for keeping the group running (smoothly). I would like to thank SSPC for funding to carry out my PhD studies; to Prof. Fergal O'Gara for providing the transaminases, and to Prof. Pat O Neill, Pfizer, for his input and giving us target molecules.

Huge thanks are due to the ARM group past and present who I have had the pleasure to work with, and learn from Fran, Alan (the questions will never end), Leslie-Ann, Denis, Niki, Aoife R, Aoife B, Amy, Aaran, Thomas, Dan, Nuala, and Rose and Laura, we were in this together from the start. Special thanks are due to Lorraine for helping me out so much from the start! Thanks to the book club, for helping me get back to my love of reading in the past year! To the rest, of which there are far too many to mention (and I fear forgetting you!), thank you! Muchas gracias a Leti para todos, te echo de menos. To Ryan and Kevin, for never saying no (but occasionally only saying maybe) to coffee. To Davy, for being brave, or stupid enough, to live and work with me! For the biocatalysis group – we're big enough to be a group now – Declan for everything since the day I started (and especially after you left!), Edel, Maria, Jerry Nacho and Dave the sincerest thanks.

I would like to sincerely thank Conor, Linda, Mary, Michelle, and Aisling for putting up with me, and to two of you for living with me in the first years! I probably would have come out a whole lot worse without you! To Rob, for knowing when to ask and when not to ask about my day, and for everything else, thank you. To my parents, grandparents, brother, and sister, for supporting and encouraging me throughout my studies! Thanks to the rest of my family, especially Moira (who knows enough to ask for a special acknowledgement, because that's the only bit everyone reads!).

To my family

Abstract

The first chapter provides an overview of the use of biocatalysis for the synthesis of pharmaceutical intermediates and natural products. This review focuses, in particular, on the impact of recent developments in technologies which enable the increased use of biocatalysts. These developments include:

- the immobilisation of biocatalysts to enhance stability and ease of use, and enabling use in combination with metal and organocatalysts leading to dynamic kinetic resolution, as well as in continuous flow.

- discovery and development of novel enzymes using molecular biology for enzyme engineering and metagenomics.

The second chapter describes the lipase-mediated kinetic resolution of 2-phenylalkanols by tuning the steric properties of the acyl group to control the efficiency and selectivity of the resolution. In contrast to literature reports, efficient resolution was achieved using short-chain acyl groups through careful process control and substrate modification. The effect of increased steric demand at the stereocentre was also explored.

Chapter three describes proof of concept that a hydrolase-catalysed dynamic kinetic resolution of a lactol is possible. By taking advantage of the spontaneous racemisation of the unreacted starting material, the dynamic kinetic resolution was carried out without the need for a separate racemisation catalyst. While the kinetic resolution was effective in a model system, the biotransformation was inhibited by the introduction of a second, remote stereocentre.

Chapter four describes a dynamic kinetic resolution of synthetically versatile nitroalcohols by combination of a lipase-mediated resolution and the reversible intramolecular nitroaldol (Henry) reaction. Significant challenges in effecting the combination of the base-mediated racemisation step and the lipase-mediated resolution step were encountered. Reaction engineering allowed design of a sequential one-pot reaction system which furnished the products with excellent enantioselectivity, and good diastereoselectivity.

Chapter five describes the use of novel transaminases in the kinetic resolution of model amine substrates and pharmaceutical intermediates by oxidative deamination, including exploration of the substrate scope of these novel biocatalysts. While transaminase-mediated reductive amination is an attractive method for asymmetric synthesis of chiral amines, the reductive amination is

generally thermodynamically disfavoured. Preliminary investigation of approaches to favour the reductive amination is described.

The final chapter contains the full experimental details, including spectroscopic and analytical data of all the compounds synthesised in this project; details of chiral phase HPLC analysis are included in the appendix.

"Creativity is combining facts no one else has connected before."

Christiane Nüsslein-Volhard, 1995 Nobel Prize in Physiology or Medicine.

Contents

Chapter 2

Resolution of 2-phenylalkanols

Contents

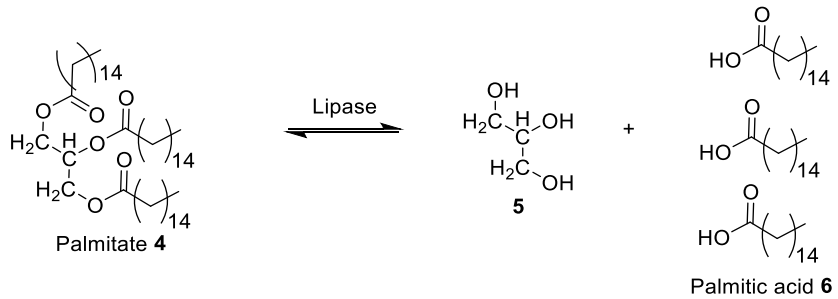
Overview

By tuning the steric properties of the acyl group to control the efficiency and selectivity of the resolution, 2-phenyl-1-propanol **1** was prepared by lipase-catalysed hydrolysis using a short-chain acyl group, with E-values of up to 66 (*ee* up to 95%). 2-Phenylbutan-1-ol **2** was similarly resolved (up to 86% *ee*) using the optimised conditions, while the ester of the more sterically demanding 3-methyl-2-phenylbutan-1-ol **3** proved resistant to enzymatic hydrolysis under these conditions.

2.1 Introduction

The use of biocatalysts in organic synthesis is a growing and attractive method for achieving chemical transformations.[1] Enzymes, by their nature, have excellent chemo-, regio- and enantioselectivity, which, if utilised correctly can give superior results to chemical catalysts.[2]

Hydrolases are the most studied biocatalysts and have found application in many industrial processes.[1] Lipases are ubiquitous enzymes belonging to the family of serine hydrolases and can be found in animals, plants, bacteria, and fungi.[3] Lipases naturally catalyse the reversible hydrolysis of the ester bonds in triacylglycerols for example **4**, producing glycerol **5** and the free fatty acids **6** (Scheme 2.1) and are highly enantioselective in the kinetic resolution of carboxylic acids, alcohols, and related derivatives. Lipases are the most frequently used enzymes in organic synthesis, due in part to being inexpensive, commercially available, and stable, but also due to their wide substrate scope and high regio-, stereo-, and chemoselectivity. The fact that lipases do not require cofactors is a significant advantage when used for biocatalysis. They are also very useful because they naturally act on the lipid water interface, thus increasing their substrate scope beyond water soluble compounds.

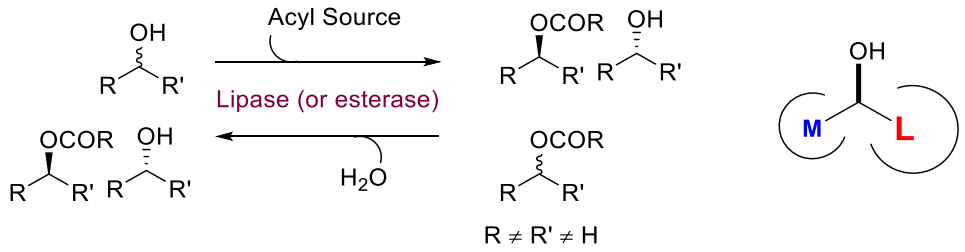


*Scheme 2.1 Lipases naturally catalyse the reversible hydrolysis of triacylglycerols such as palmitate **5***

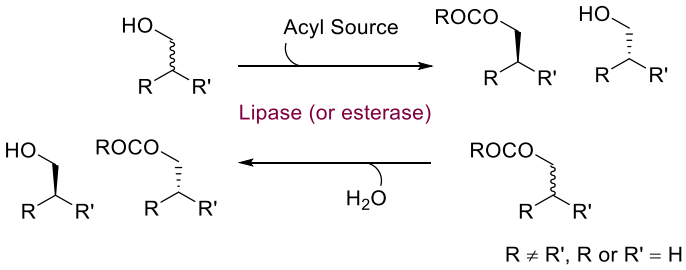
Many different strategies can be employed to increase the selectivity of the lipases including, but not limited to: addition of organic solvents,[4-6] changing temperature,[7,8] immobilisation and modification of the enzyme,[9,10] the presences of additives,[11-13] and, in the case of (trans)esterification, modification of the acyl donor.[14-20]

Studies on the selectivity of lipases usually focus on compounds where the chiral centre is adjacent to the site of reaction, e.g. α-alkyl carboxylic acids or secondary alcohols (Scheme 2.2). Secondary alcohols are so well studied that there are size based rules for predicting stereochemical outcome with well-known lipases.[1] Extension of these systems, where the

chiral centre is more remote, e.g β-or γ-alkyl carboxylic acids or primary alcohols (Scheme 2.3), can lead to challenges in terms of efficiency and selectivity, and they are therefore less studied than their secondary alcohol counterparts.[21-24]



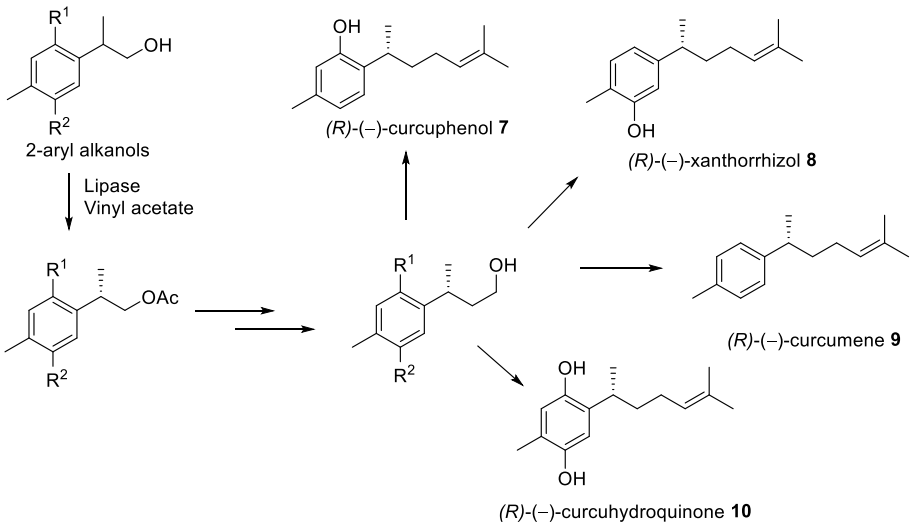
Scheme 2.2 Hydrolysis and transesterification of chiral secondary alcohols (left), size-based rules for predicting stereochemical outcome (right)



Scheme 2.3 Hydrolysis and transesterification of primary alcohols

2.1.1 Importance of 2-phenylalkanols as synthetic intermediates

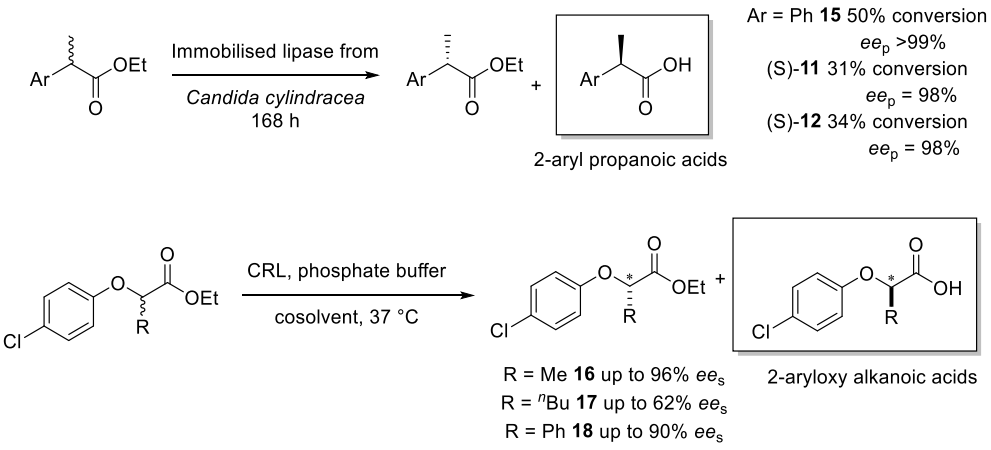
Chiral primary alcohols can be used as building blocks in many drugs, such as the profenols, but are also important in the flavour industry and for the synthesis of natural products including **7–10** (Scheme 2.4).[25]



Scheme 2.4 Synthesis of natural products from 2-aryl alkanols

Chiral primary alcohols, having a benzylic stereocentre, can be used in the synthesis of 2-arylpropionic acids, a common class of non-steroidal anti-inflammatory drugs (NSAIDs) (Scheme 2.5), [26,27] as well as in the synthesis of fragrance molecules,[28,29] and substituted coumarins.[25] In most cases, only one form (*R* or *S*) of the drug is active, but the drug may still be produced and sold racemically. For example, *(S)*-ibuprofen *(S)*-**11** is pharmacologically active but it is sold as a racemic mixture. *(S)*-Naproxen *(S)*-**12** was the first NSAID marketed as a single enantiomer.[30] For fragrance or flavour molecules, the different enantiomers can have different smells or tastes e.g *(R)*-(–)-carvone (spearmint) **13** and *(S)*-(+)-carvone (caraway) **13**.[31] The fragrance of Pamplefleur **14** changes only when the stereocentre at C2 changes.[28,29]

Previous work involving 2-aryl and 2-aryloxy propionic acids (being classes of non-steroidal anti-inflammatory drugs, and herbicides, respectively, (Scheme 2.5) achieved the resolution of esters **11**, **12** and **15-18** bearing the stereocentre at the α-position,[32-37] where the conditions were optimised using cosolvents, and immobilising the lipases, as well as mechanistic studies to determine the best conditions for the reactions.

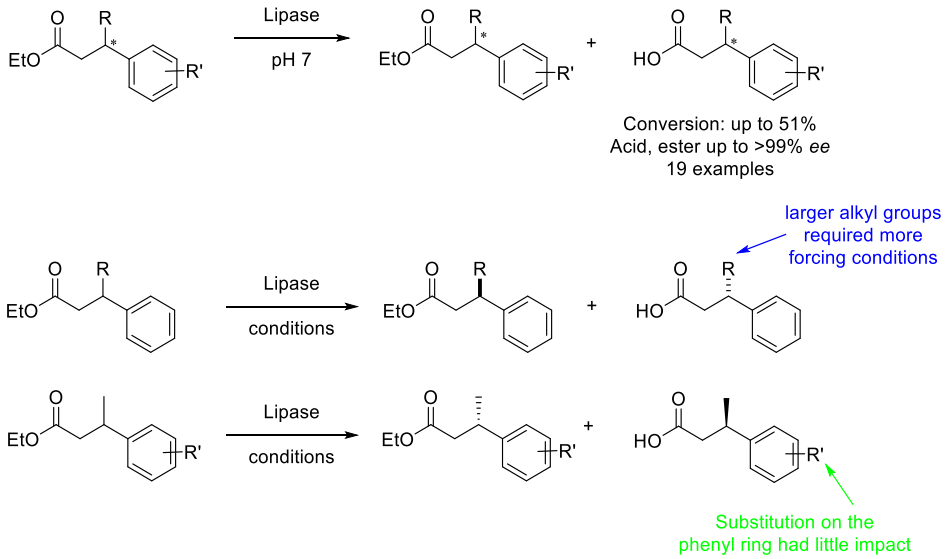


Scheme 2.5 Resolution of 2-aryl and 2-aryloxyalkanoic acid esters

2.1.2 Previous work in our group

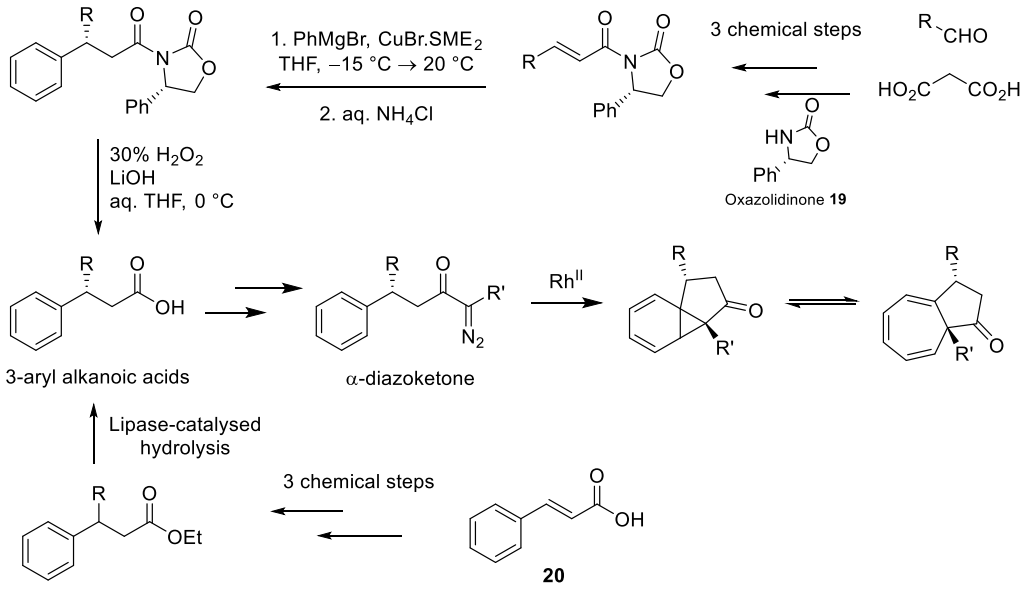
Previous work within our group on the lipase mediated kinetic resolution of chiral 3-aryl alkanoic acids, bearing the stereocentre on the carboxylic acid moiety, showed hydrolase recognition of stereocentres β to the reacting site (Scheme 2.6).[38,39] While under standard conditions, the outcomes were modest, excellent enantioselectivities were achieved for both the ester substrate and acid product through extensive process optimisation. This study showed the effect of changing substitution on the chiral centre, by systematically varying first the alkyl group, followed by the aryl group; as the size of the alkyl group was increased, the

resolution required more forcing conditions in order to hydrolyse the ester.[38,39] The electronic effects through substitution of the aromatic ring were also investigated;[39] this showed that the effect of the aryl substitution on the resolution was minimal, having little effect on the outcome of the resolution.



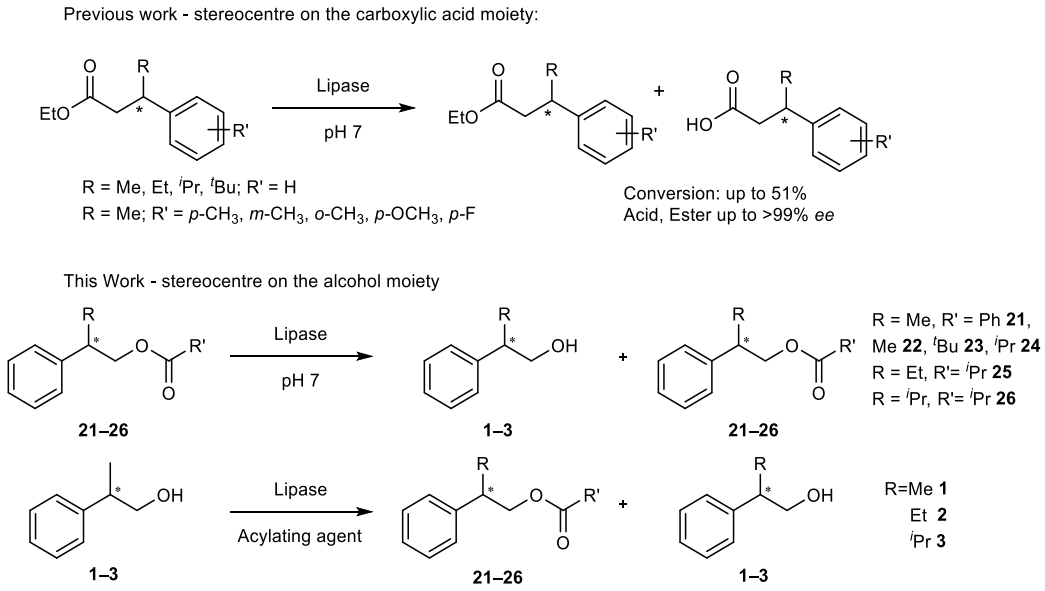
Scheme 2.6 Resolution of carboxylic acids containing remote stereocentres – the effect of substitution.

These compounds are synthetically important within our group; enantiopure 3-aryl alkanoic acids are precursors to α-diazoketones, which are used as substrates in the Buchner cyclisation reaction (Scheme 2.7). The reaction has been shown to proceed with very high diastereoselectively when using an enantiopure carboxylic acid starting material (Scheme 2.7).[40,41] The development of enzymatic methods to resolve compounds of this type,[38,39] could replace the use of the chiral oxazolidinone **19** directing group when accessing these compounds. Advantages of this include the use of a readily available carboxylic acid starting material **20**, the removal of the need to add and remove the chiral auxilary, as well as the use of mild conditions (Scheme 2.7).



Scheme 2.7 Buchner cyclisation

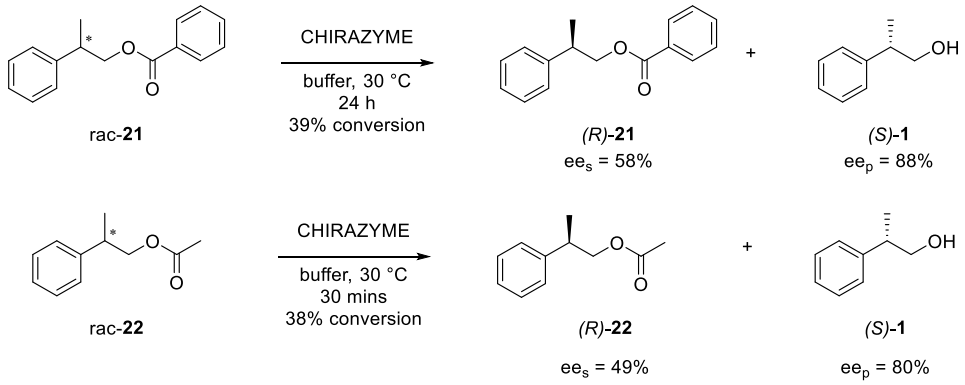
Extension to this work, using derivatives with the chiral centre on the alcohol moiety, removed from the reacting site is the focus of this project. Both the hydrolysis and transesterification reactions can potentially provide access to a series of enantioenriched alcohols and esters (Scheme 2.8). This work focuses on the exploitation of the excellent chemo, regio- and enantioselectivity associated with lipases in the synthesis of 2-phenyl-1-propanol **1**, studying both transesterification and hydrolysis reactions of 2-phenyl-1-propanol **1** and derivative esters **21–24**, respectively, as well as derivative alcohols containing larger alkyl groups **2** and **3** and esters thereof **25** and **26** (Scheme 2.8).



Scheme 2.8 Hydrolysis and transesterification of chiral primary alcohols and esters

2.1.3 Previous enzymatic resolution of 2-phenyl-1-propanol **1**

The resolution of 2-phenyl-1-propanol **1** has previously been reported, where good selectivities and conversions were attained, using both 2-phenylpropyl benzoate **21** (up to 88% ee_p, E = 29) (Scheme 2.9), and 2-phenylpropyl acetate **22** (up to 80% *ee,* E = 14).[17] In general, high enantioselectivity can be achieved in the hydrolysis of 2-phenyl-1-propanol **1** but with the disadvantage of using larger acyl chains (some examples **27–29** shown in Figure 2.8).[17,18,25,42-45] While these chains have the advantage of conferring excellent selectivity, removing the carboxylic acid by-products could lead to problems. The study of short chain acyl groups, aside from acetate, is uncommon in this specific resolution; very few examples are reported.[24,46,47] In the case of smaller carboxylic acid groups, they are usually removed in the work-up and are left in the aqueous layer, leaving only a two-component system in the organic layer, giving an easier separation. In this work small, alkyl groups are used, which is more atom economical in addition to the more convenient work-up.



*Scheme 2.9 Lipase-catalysed resolution of **21** and **22***

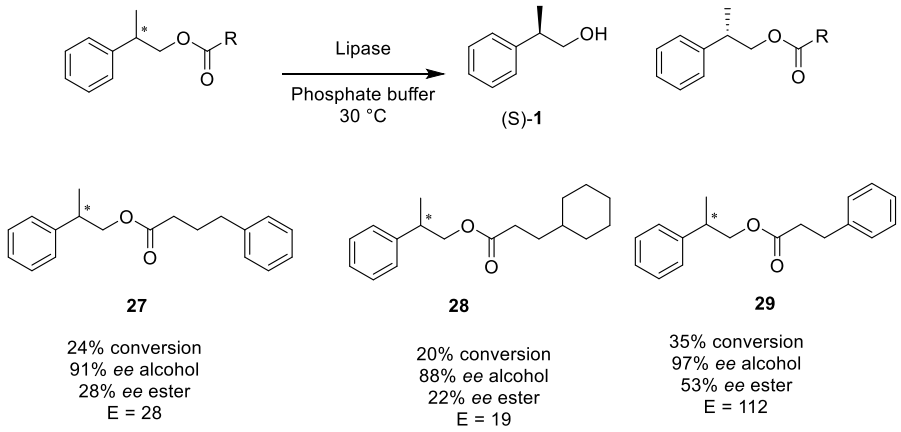
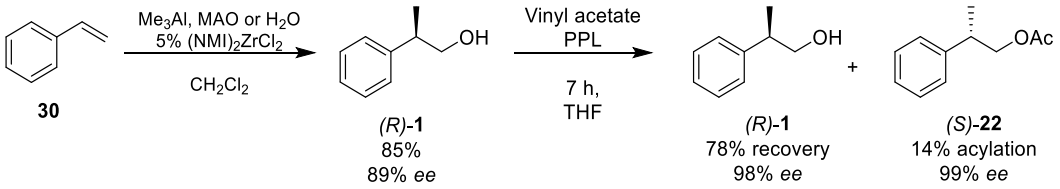


*Figure 2.8 Product esters from the transesterification of 2-phenyl-1-propanol **1** using long chain acyl chains*

For transesterification of 2-phenyl-1-propanol **1**, the acylating agent has been extensively studied, including the effect of a phenyl group on the resolution (phenyl group at β-position is favourable), the length of the alkyl chains (longer chains tend to give better selectivity), and the electronic effects of the acyl chain (with isomeric pentenoic acids as acyl donors it was shown that the position of the double bond is important).[14,18]

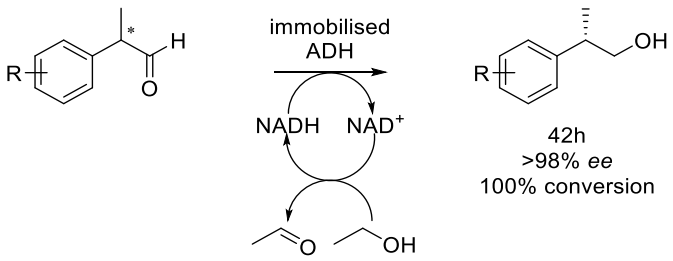
2.1.4 Enantioselective synthesis of 2-phenyl-1-propanol **1** – other chemoenzymatic methods

2-Phenyl-1-propanol **1** has previously been prepared enantioselectively from styrene, followed by a lipase catalysed transesterification to further enhance *ee* (Scheme 2.10).[48]



Scheme 2.10 Use of porcine pancreas lipase (PPL) to enhance ee

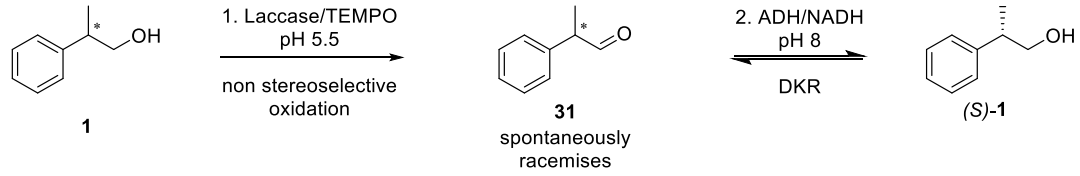
There have been several reports of resolution of racemic alcohols using biocatalysts as shown in Scheme 2.11.[49,50] The mixture is racemised on oxidation, and resolved on reduction. However, while this is a dynamic kinetic resolution, theoretically giving 100% conversion, a disadvantage is that a cofactor is required, and with that, a cofactor regeneration system.



Scheme 2.11 Resolution of chiral primary alcohols using alcohol dehydrogenase (ADH) as biocatalyst

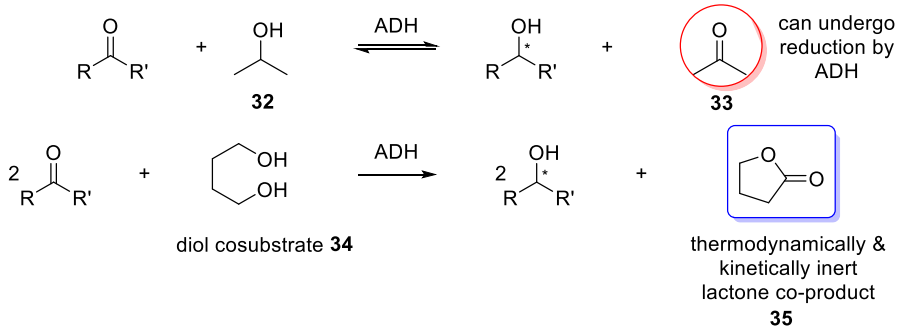
Gotor et al. reported the deracemisation of 2-phenyl-1-propanol **1** by non-selective oxidation of the racemic alcohol to the aldehyde, which undergoes spontaneous racemization, using laccase from *T. versicolor* and TEMPO as an oxidant, followed by enantioselective reduction using an immobilized alcohol dehydrogenase (ADH) (Scheme 2.12).[51] Laccase is a biocatalyst which utilizes molecular oxygen as an oxidant. The enzymes used are active at different pHs.

Both enantiomers were accessible (>94% *ee*) through the use of stereocomplimentary enzymes.



*Scheme 2.12 One-pot stereoselective synthesis of 2-phenyl-1-propanol **1***

The advantage of this synthesis is the use of a cheap racemic starting material. However, because an oxidoreductase (ADH) is employed a cofactor is also required, which is regenerated by the addition of ethanol or 2-propanol **32** as a co-substrate (Scheme 2.11). Smart cosubstrate selection, such as diol **34**, can improve the efficiency of reactions of this type by producing an inert by-product **35** (Scheme 2.13).[52]

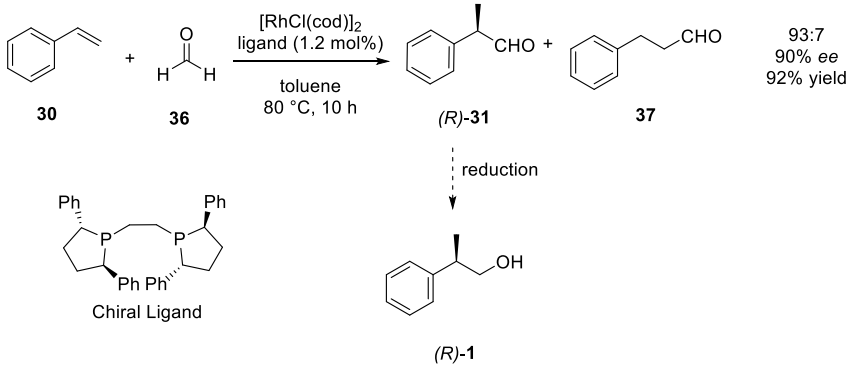


Scheme 2.13 Smart co-substrate selection can improve the conversion of the reactions

2.1.5 Non-chemoenzymatic synthesis of enantiopure 2-phenyl-1-propanol **1**

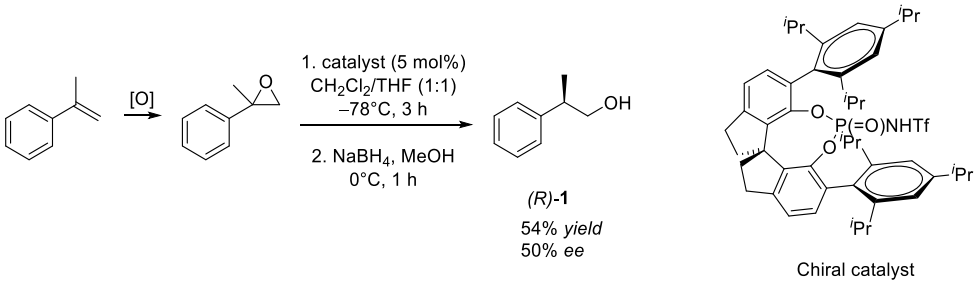
There are a number of recently reported routes to substituted β-aryl alcohols. There are several reports of non-stereoselective reduction of the corresponding aldehydes, catalysed by iron, immobilised ruthenium, and silver.[53-55] Beller et al. reported the synthesis using two ruthenium catalysts cooperatively.[56] Ley et al. prepared a series of racemic alcohols from terminal alkenes through a continuous flow hydroboration-oxidation reaction.[57]

Stereoselective synthesis of aldehydes from terminal alkenes has been reported, giving the internal aldehyde *(R)*-**31** in a stereoselective fashion (up to 95% *ee*) with only minor amounts (7%) of the regioisomer **37**. Reduction of *(R)*-**31** could allow access to the alcohol *(R)*-**1** (Scheme 2.14).[58]



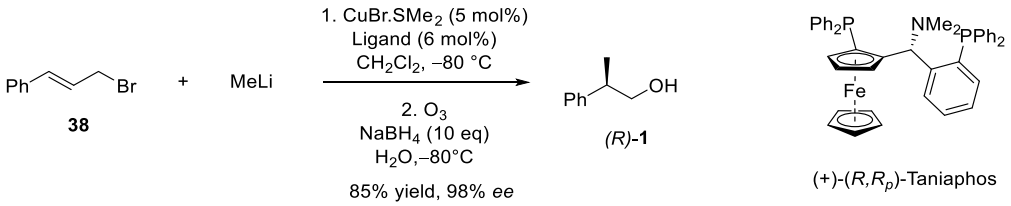
*Scheme 2.14 Asymmetric hydroformylation to give aldehyde (R)-**31***

Zhuang and Du reported a one pot, stereoselective synthesis of 2,2-substituted alcohols from alkenes through a sequential epoxidation, Brønsted-acid catalysed rearrangement to a chiral aldehyde, and reduction with retention of stereochemistry, which gave up to 50% *ee* (Scheme 2.15).[59]



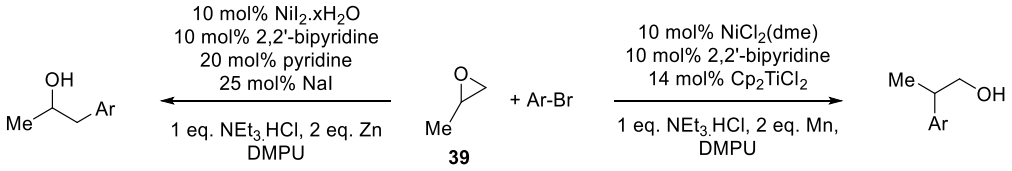
Scheme 2.15 Asymmetric rearrangement of racemic epoxides

Feringa et al. reported a copper catalysed asymmetric allylic alkylation of **38**, using organolithium reagents, followed by either reductive ozonolysis, which furnished the 2-substituted alcohols (up to 99% *ee*) (Scheme 2.16), or hydroboration-oxidation, which extended the chain by one carbon, and gave 3-substituted alcohols.[60]



Scheme 2.16 Copper-catalysed asymmetric allylic alkylation

Zhao and Weix reported the nickel catalysed coupling of epoxide **39** and arylhalides. The regiochemical outcome of this coupling can be controlled by an additive, either iodide or titanium (Scheme 2.17).[61]



Scheme 2.17 Regiochemistry controlled by iodine or titanium additives

Each of the above stereoselective reactions involved the use of complex chiral ligands to induce stereoselectivity. Biocatalytic routes to enantiopure or enantioenriched compounds would avoid the use of complex chiral ligands or metal catalysts, as well as extremes of temperature and the use of organic solvents.

2.2 Objectives of this project

The overall objective of this project is to explore the effect of variation of the acyl group on the resolution of 2-phenyl-1-propanol **1**, focusing specifically on the effect of smaller acyl chains which are poorly represented in the literature.

The specific objectives of this study can be summarised as follows:

- To prepare a series of esters of 2-phenyl-1-propanol **1**.
- To develop chiral HPLC conditions which will separate both enantiomers of the alcohol and the ester
- To screen these esters against a library of lipases in order to assess their activity in the resolution of 2-phenyl-1-propanol **1** by variation of the following factors:
    - Variation of biocatalyst.
    - Alteration of the ester group.
    - Changing the mode of reaction.
    - Alteration of the reaction conditions (e.g. temperature, cosolvent addition, reaction time).
- To conduct a preparative scale synthesis of 2-phenyl-1-propanol **1**.
- To confirm the absolute stereochemistry of 2-phenyl-1-propanol **1**.

2.3 Synthesis of substrates

The aim of this section of the project was to synthesize a series of chiral esters to test against lipase enzymes. The compounds varied by having different ester groups (R′), as well as different substituents (R) at the β-position of that ester (Figure 2.9)

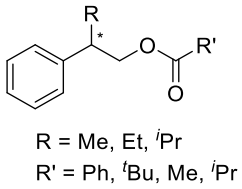


*Figure 2.9 General structure of the target ester substrates **21 – 26***

The key starting materials for the synthesis of the ester substrates are chiral primary alcohols, 2-phenylpropan-1-ol **1**, 2-phenylbutan-1-ol **2** and 3-methyl-2-phenylbutan-1-ol **3**, Figure 2.10, of which two are commercially available, **1** and **2**. Alcohol **3** is not commercially available, and was synthesised for this investigation

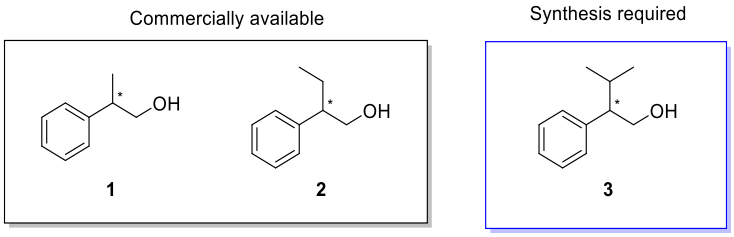
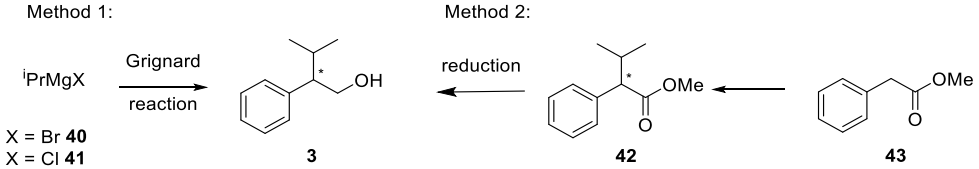


*Figure 2.10 Starting alcohols **1–3**.*

2.3.1 Alcohol synthesis

3-Methyl-2-phenyl-1-butanol **3** was synthesized by two methods (Scheme 2.18). The first method used a Grignard reaction, and the second approach used a substitution reaction followed by a reduction.



*Scheme 2.18 Synthesis of 3-methyl-2-phenylbutan-1-ol **3***

2.3.1.1 Method 1 – Grignard reaction

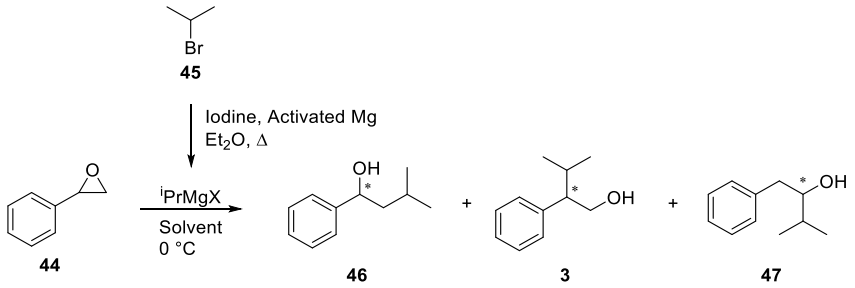
The first attempted route was through a Grignard reaction, using styrene oxide **44** as the electrophile, and in situ generated isopropyl magnesium bromide **40** as the nucleophile.[62]

The reaction uses approx. 1.2 equivalents of magnesium and 1.1 eq. of the alkyl halide to prepare the Grignard reagent. The disappearance of styrene oxide **44** by TLC is used to monitor the reaction progress and is generally complete within 30 minutes.

There are many reported methods to enhance the efficiency of the initiation of the Grignard reaction.[63-65] In this case the magnesium was activated before use by vigorous stirring in ~1 mL of solvent in the presence of a crystal of iodine for 1–1.5 h. To initiate the reaction, the neat alkyl halide **45** was added slowly to the solution, until the reaction had started, which is evident by a colour change from brown to grey. Once the reaction was initiated, the alkyl halide was diluted with the required amount of solvent for the reaction and added at such a rate as to maintain reflux without external heating. As the presence of even minor amounts of water has a detrimental effect on Grignard reagents freshly opened bottles of diethyl ether were used in this reaction.[63,66-69]

The addition of the Grignard reagent to the styrene oxide can give rise to two isomeric products. However, the second expected product **46**, was not observed; instead a rearrangement product **47** was isolated (Table 2.1). The reaction was also carried out using commercially available isopropyl magnesium chloride **41** (2.0 M in THF), in this case the ratios of products formed were different, this is due to solvent induced change in the Schlenk equilibrium. This reactivity has been previously reported and the product ratios can be altered by adding the Grignard reagent to the epoxide instead of adding the epoxide to the Grignard reagent.[70,71]

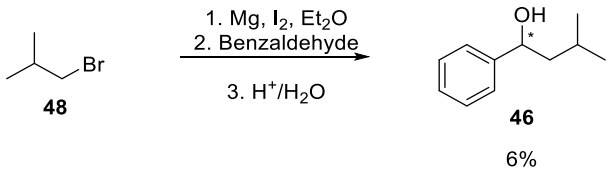
*Table 2.1 Preparation of alcohol **3***



Entry	X	Solvent	Ratio 46:3:47	Yield 3 (%)	Yield 47 (%)
1	Br (in situ generated)	Et_2O	0:45:55	21	21
2[a]	*Cl* (commercially available, 2.0M in THF)	THF, distilled	0:66:34	30	12[b]

[a]Carried out by fourth year student; [b]material was recovered from the column, made up of compound **3** and **47** (63:37) coeluting, and was equivalent to approximately 25% yield, it was not separated as sufficient material was isolated.

Figure 2.11 shows the ^{1}H NMR spectrum of the crude material from the Grignard reaction using the commercially available Grignard reagent **41**. The spectra of the two expected products **46** and **3** are also shown. Alcohol **46** was synthesised independently by a Grignard reaction which would not give rise to isomeric products (Scheme 2.19); the reaction was low-yielding due to incomplete formation of the Grignard reagent; a freshly opened bottle of benzaldehyde was used but it was not distilled. Sufficient material was isolated for analysis. The absence of the 1H dd at 4.75 ppm, corresponding to the proton at the chiral centre of **46** (C**H**OH) indicates that this product was not formed in the Grignard reaction using styrene oxide **44**.



*Scheme 2.19 Synthesis of **46***

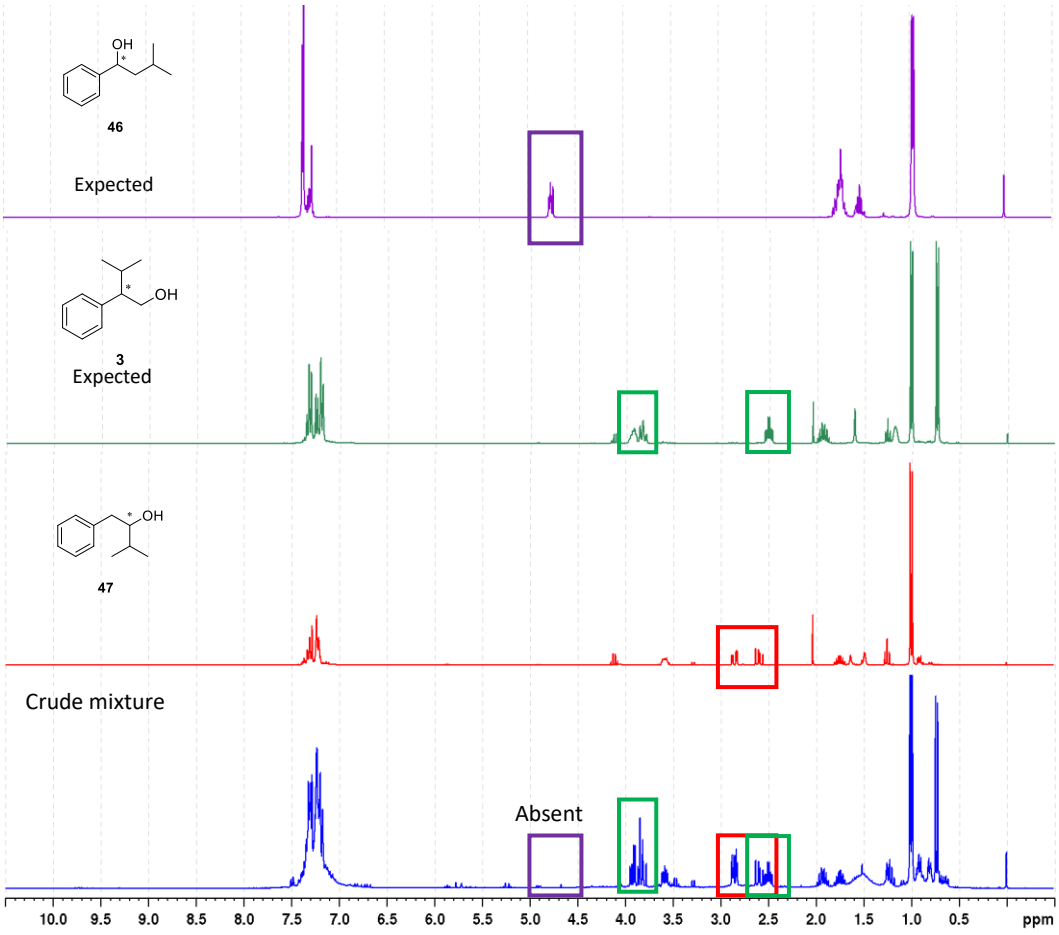
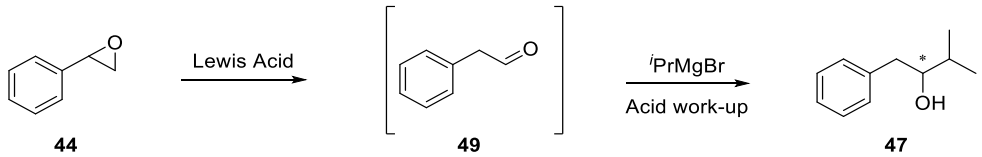


*Figure 2.11 ^{1}H NMR spectra, showing the crude material (blue), the rearrangement product **47** (red), the target alcohol **3** (green), and the expected regioisomer **46** (purple). ($CDCl_3$, 300 MHz)*

The product formed was analysed by ^{1}H and ^{13}C NMR and DEPT experiments; 2D experiments were carried out in order to identify the product **47**. It is thought that the product arose from a Lewis acid catalysed ($MgBr_2$) Meinwald rearrangement, via aldehyde intermediate **49** (Scheme 2.20).[70] Data for **47** matches previously reported data.[72] All alcohol products are known compounds and spectroscopic data is consistent with previous reports.



Scheme 2.20 Meinwald rearrangement

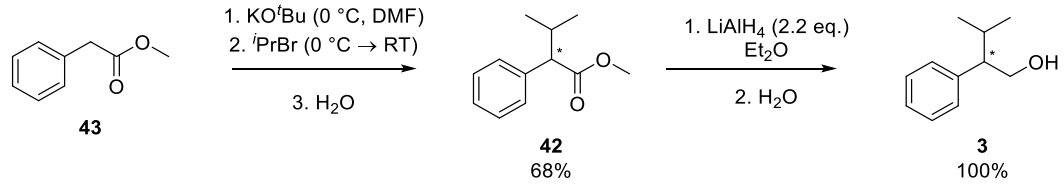
The rearrangement of epoxides in the presence of Lewis or Brønsted acids has been previously reported.[59,73-75] In particular the rearrangement of styrene oxide under Grignard conditions

has been reported, and the regiochemical outcome can be modified by changing the order of addition of reagents i.e. adding styrene oxide **44** to the Grignard reagent can give different ratios of regioisomers to adding the Grignard reagent to the styrene oxide.[70,71]

The yield of the desired alcohol **3**, after column chromatography, was 21%. While this could be scaled up to provide a reasonable amount of material from cheap starting materials, it is inefficient, and so another route was explored.

2.3.1.2 Alternative route

The synthesis of alcohol **3** via a known synthetic route was carried out in accordance with previously reported procedure (Scheme 2.21).[76]



43 | **42** 68% | **3** 100%

*Scheme 2.21 Alternative route to alcohol **3***

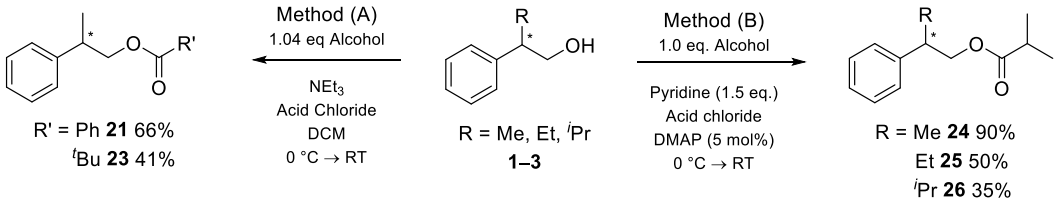
The first step involved deprotonation of the methyl 2-phenylacetate **43** by potassium *tert*-butoxide in DMF, and the subsequent alkylation using isopropyl bromide **45**. Complete removal of the DMF required extensive washing, as described in the experimental section. After column chromatography, **42** was reduced using lithium aluminum hydride, according to literature procedure, and required no purification.[76] Using this method, multigram quantities of alcohol **3** could be readily prepared.

2.3.2 Ester synthesis

The initial route to the esters, used in the synthesis of **21**, required the use of an excess of alcohol (1.2 equivalents) in the presence of the appropriate acid chloride. When this amount was reduced (to 1.04 eq.) the efficiency of the reaction dropped (Scheme 2.22, method A). In the synthesis of 2-phenylpropyl benzoate **21**, this resulted a slightly decreased yield from 70% to 66%.

The introduction of a nucleophilic catalyst, DMAP, increased the efficiency of the reaction, utilising only one equivalent of the alcohol and, in general, gave complete conversion to the ester (Scheme 2.22, method B).[77] The products obtained were of excellent purity, but were

subjected to column chromatography as very pure material was needed for the enzymatic reactions.



Scheme 2.22 Ester synthesis

2.4 Hydrolase-mediated kinetic resolution – analytical screens

2.4.1 Screening protocol – hydrolysis of esters

The hydrolysis screening reactions were carried out at approx. 50 mM scale (50 mg of ester in 4.5 mL buffer). The reaction mixture was subjected to a mini work-up, and filtered through Celite®. No drying agent was used. If conversion was detected, the crude mixture was analysed by chiral HPLC analysis. Purification was not necessary before HPLC analysis, as the reactions produce no by-products. The crude material contained only ester substrates and alcohol product. This allowed us to carry out the screening reactions on a small scale; this reduced the amount of material necessary for screening. Generally, when both the alcohol and the ester separated on a given chiral column, then other esters of the same alcohol would also separate on that column.

Transesterification reactions were carried out in neat vinyl ester (0.5 mL) using 150 mM (10 mg) of 2-phenyl-1-propanol **1**. The reaction solution was simply filtered through a plug of Celite® before analysis, and analysed like the hydrolysis reactions.

2.4.2 Screening – initial target 2-phenylpropyl benzoate **21**

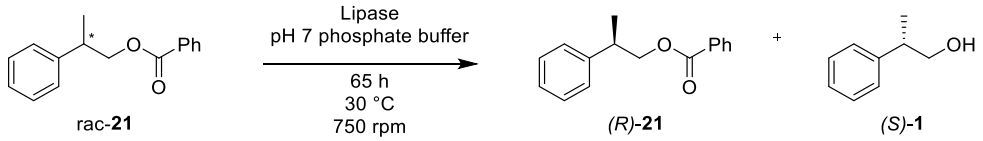
Ester **21** was initially screened against a targeted panel of lipases supplied by Almac in a previous study carried out by Gavin.[78] Of the 52 enzymes tested, 15 gave no conversion, 22 gave conversion under 10%, 4 gave 100% conversion; a selection of the results are shown in Table 2.2 (Entries 1–11). Using the lipase from *Pseudomonas cepacia*, excellent enantioselectivity toward the alcohol **1** was achieved (93% *ee*) (Entry 4). Lipases C, D, E and F from *Alcaligenes sp.* showed similar selectivities towards the product, with similar E values (Entries 7, 9, 2 and 10, respectively).

When the work was progressed in this study, a small selection of commercially available lipases was screened for activity towards the chosen substrate (Table 2.2, Entries 12–16) and subsequently used for other substrates. The commercially available lipases used were: Hog pancreas lipase, CAL-B (immob), *Pseudomonas fluorescens* (immob), Amano PS Lipase, and Lipase from *Candida cylindracea.*

During this study the lipase from *Candida cylindracea (*Table 2.2*,* entry *15)* gave a good conversion but without selectivity. This hydrolysis was subjected to a small solvent screen, including 1-octanol (Table 2.2, entry 16), which furnished us with ee_p = 27%, ee_s = 20%, c =

42%. While this is poorly selective overall, it does show a drastic increase in selectivity compared to the absence of the organic cosolvent (Entry 15). While the use of *Pseudomonas fluorescens* (Entry 3) gave 30% conversion with moderate enantioselectivity, the use of the immobilised lipase (Entry 13) gave <5% conversion.

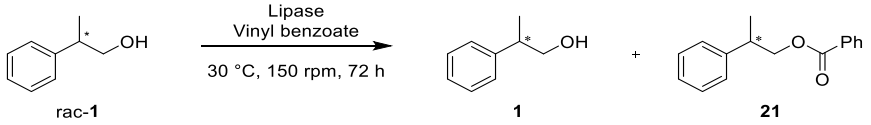
*Table 2.2 Hydrolysis of 2-Phenyl-1-propyl Benzoate **21***



Entry	Lipase	Conversion (%)		*ee*[a] (%)		E
		NMR	E_{calc}	ee_s	ee_p	
1	Lipase A from *Burkholderia cepacia*	47	48	66	71	11
2	Lipase E from *Alcaligenes sp*	30	33	23	46	3.4
3	Lipase from *Pseudomonas fluorescens*	29	29	28	69	7.1
4	Lipase from *Pseudomonas cepacia*	23	32	44	93	42
5	Lipase from *Aspergilus niger*	22	24	8	26	1.8
6	Porcine *Pancrease* type II	17	19	7	30	2
7	Lipase C from *Alcaligenes sp*	15	32	18	39	2.7
8	*Candida antartica* lipase B (immob)	15	19	21	87	17
9	Lipase D from *Alcaligenes sp.*	14	11	8	64	4.9
10	Lipase F from *Alcaligenes sp.*	11	7	4	57	3.8
11	Lipase from *Candida antarctica*	10	18	20	89	20
12	Hog pancreas lipase	4	-[b]	-[c]	-[c]	-[b]
13	*Pseudomonas fluorescens* (immob)	2	-[b]	-[c]	-[c]	-[b]
14	Amano PS lipase	9	-[b]	-[c]	-[c]	-[b]
15	Lipase from *Candida cylindracea*	48	-[d]	0	0	-[d]
16	Lipase from *Candida cylindracea* with 17% *v/v* 1-octanol	-[e]	42	27 *(R)*	20	2

Entries 1 to 11 were carried out by D. Gavin. [a]Enantiomeric excess values determined by chiral HPLC analysis; [b]E_{calc} and E values were not determined, as *ee* values were not measured when conversion <10%; [c]enantiomeric excess values were not determined when coversion was <10%; [d]E_{calc} and E values were not determined, as enantioselectivity was <1% *ee;* [e]conversion was not determined by ^{1}H NMR due to the presence of 1-octanol which was not effectively removed by rotary evaporation because of high b.p. (195°C).

The resolution was also attempted using the transesterification reaction (Scheme 2.23), as the selectivity can be changed by changing the mode of reaction.[79] Unfortunately, the transesterification reaction with vinyl benzoate gave no conversion when Hog Pancreas lipase, CAL-B (immob), *Pseudomonas fluorescens* (immob), or Amano PS Lipase were used; Lipase from *Candida cylindracea* gave 100% conversion after 72 hours.



*Scheme 2.23 Transesterification of **1** using vinyl benzoate*

Lipase from *Candida cylindracea* does not give selectivity for our compounds. It has been used for transesterification of secondary alcohols to esters having alkyl chains on the acyl moiety and for transesterification of secondary amines.[80,81]

2.4.3 Screening – small acyl group: acetate

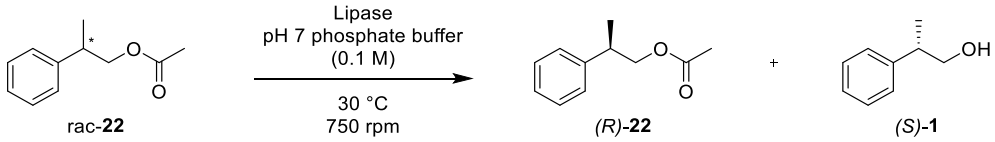
To explore the steric demands of the acyl group, it was decided to test the commercial enzymes against a much smaller ester group, the acetate group. Resolution by hydrolysis has previously been reported but was poorly selective (E = 4) for 2-phenyl-1-propyl acetate **22**.[82] More common however, is the resolution of the acetate **22** by transesterification.[25,44,83,84]

Resolution of 2-phenyl-1-propanol **1** was attempted using both hydrolysis and transesterification reactions, and the reaction times were adjusted to ensure the optimum kinetic resolution conversion ~50%. Five lipases tested gave >50% hydrolysis product after 65 hours (Table 2.3 and Table 2.4, additional timepoints are shown in **Appendix III**).

The presence or absence of molecular sieves had little effect on the transesterification reactions, with molecular sieves increasing the conversion, but having very little effect on the E value.[21]

Following these experiments, and with reference to the literature,[17,25,44,82,83,85] it was clear that the acetate group was not bulky enough to give good enantioselectivity at 30°C. One option would be to carry out the reaction at a lower temperature, which is known to aid selectivity. Looking at the time screens, where lower reaction times did not necessarily furnish better selectivity, it was decided to change the ester to a bulkier group.

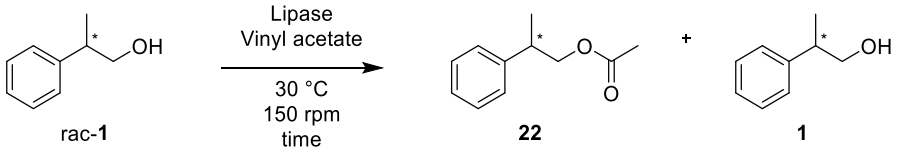
*Table 2.3 Hydrolysis of 2-phenylpropyl acetate **22***



Entry	Lipase	Time (h)	Conversion (%)		*ee*[a] (%)		E
			NMR	E_{calc}	ee_s	ee_p	
1	Hog pancreas lipase	65	61	55	65	54 *(S)*	6
		48	56	56	71	55 *(S)*	7
2	*Candida antarctica* lipase B (immob)	65	87	62	26	16 *(S)*	2
		24	55	54	72	61 *(S)*	9
		18	56	57	73	55 *(S)*	7
3	*Pseudomonas fluorescens* lipase (immob)	65	82	62	10	6 *(S)*	1
		24	52	53	17	15 *(S)*	2
4	Amano PS lipase	65	100	-	-	-	-
		6	57	57	25	19 *(S)*	2
5	Lipase from *Candida cylindracea*	65	87	60	3	2 *(R)*	1
		48	56	47	4	3 *(R)*	1
		6	42	38	3	5 *(R)*	1

In the absence of an enzyme, the reaction gave no product after 48 hours. [a]Enantiomeric excess values were determined by chiral HPLC analysis.

*Table 2.4 Transesterification of 2-phenyl-1-propanol **1** using vinyl acetate as acyl source*



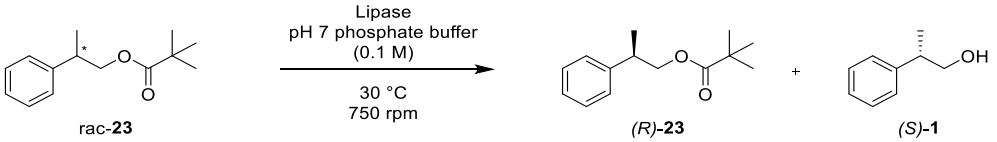
Entry	Lipase	Time (h)	Conversion (%)		*ee*[a] *(%)*		E
			NMR	E_{calc}	ee_s	ee_p	
1	Hog pancreas lipase	24	78	79	99 *(R)*	27	7
		24[b]	86	91	99 *(R)*	10	4
		18	58	57	85 *(R)*	63	11
2	*Candida antarctica* lipase B (immob)	24	100	-[c]	-[c]	-[c]	-[c]
		24[b]	100	-[c]	-[c]	-[c]	-[c]
		2	52	67	2 *(R)*	1	1
3	*Pseudomonas fluorescens* lipase (immob)	24	99	-[c]	-[c]	-[c]	-[c]
		24[b]	100	-[c]	-[c]	-[c]	-[c]
		2	46	43	4 *(R)*	5	1
4	Amano PS lipase	24	79	80	45 *(R)*	11	2
		24[b]	95	95	70 *(R)*	4	2
		6	44	45	16 *(R)*	20	2
5	Lipase from *Candida cylindracea*	24	100	-[c]	-[c]	-[c]	-[c]
		24[b]	11	9	1	10	1

Lipase from *Candida cylindracea* gave conversion > 80% even after only 2 hours (E = 1). Reactions carried out in the absence of a lipase, gave no product after 24 hours. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]carried out in the presence of 4Å molecular sieves; [c]when conversion was 100%, enantiomeric excess was not measured, and E_{calc} and E were not determined.

2.4.4 Screening – larger acyl group: pivalate

The pivalate ester, 2-phenylpropyl pivalate **23**, was synthesised and screened against the lipases for hydrolysis and transesterification. The hydrolysis reactions gave excellent enantioselectivity towards the alcohol **1**, when Amano PS Lipase was used, albeit coupled with poor conversion (Table 2.5, Entry 4). Other lipases tested gave little to no conversion; those which gave conversion showed poor enantioselectivity for the product **23**.

*Table 2.5 Hydrolysis of 2-Phenylpropyl pivalate **23***



rac-**23** | *(R)*-**23** | *(S)*-**1**

Entry	Lipase	Conversion (%)		*ee*[a] *(%)*		E
		NMR	E_{calc}	ee_s	ee_p	
1	Hog Pancreas lipase	3	29	4	10 *(S)*	1
2	*Candida antarctica* lipase B (immob)	0	-[b]	-[c]	-[c]	-[b]
3	*Pseudomonas fluorescens* lipase (immob)	0	-[b]	-[c]	-[c]	-[b]
4	Amano PS lipase	15	13	14	90 *(S)*	22
5	Lipase from *Candida cylindracea*	9	11	0	8 *(R)*	1

Reaction carried out using no enzyme gave no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]E_{calc} conversion and E were not determined as this requires *ee* > 1; [c]enantiomeric excess values were not determined as conversion was 0%.

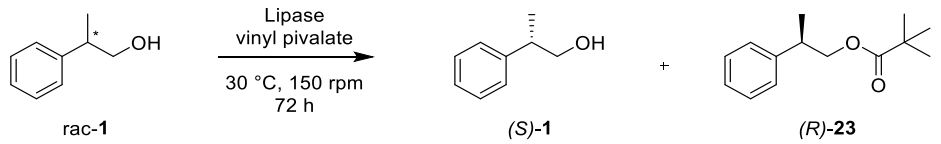
Transesterification reactions (Table 2.6) were poorly selective, giving up to 62% enantiomeric excess of the ester **23**. *Candida antarctica* lipase B (immobilised) gave complete conversion in the reaction time (72 h).

The hydrolytic reaction catalysed by Amano PS Lipase was subjected to a solvent screen (Table 2.7). The temperature was increased to 50 °C, as the lipase is stable to this temperature, and the conversion in the absence of a cosolvent was low; it was hoped that the conversion would increase with increased temperature.

The increased temperature gave a slight decrease in the enantioselectivity towards the alcohol **1** (Table 2.7, Entry 1 vs. Table 2.5, Entry 4). A moderate increase in conversion was observed in the absence of a cosolvent at the higher temperature. The addition of cosolvents gave excellent enantioselectivity, albeit with very poor conversion. It should be noted that in some cases the conversion could not be calculated, as this requires the % *ee* to be >1%; similarly, the E value could not be determined when using methyl *tert*-butyl ether, *n*-heptane and acetonitrile as solvents.

At this stage, the reaction time could have been extended but this process would not be synthetically useful, as the extended time would make the process unattractive. It was decided to try a different substrate.

*Table 2.6 Transesterification of **1** using vinyl pivalate*

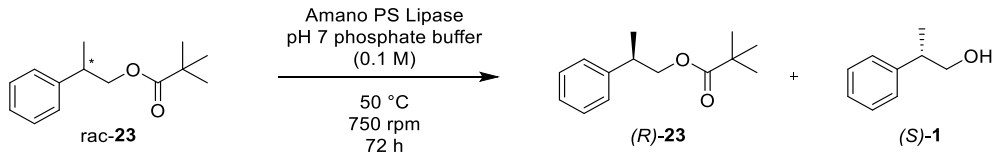


rac-**1** (*S*)-**1** (*R*)-**23**

Entry	Lipase	Conversion (%)		*ee*[a] (%)		E
		NMR	E_{calc}	ee_s	ee_p	
1	Hog Pancreas lipase	0	-[b]	-[c]	-[c]	-[b]
2	*Candida antarctica* lipase B (immob)	100	-[b]	-[d]	-[d]	-[b]
3	*Pseudomonas fluorescens* lipase (immob)	37	33	31 *(R)*	62	6
4	Amano PS lipase	8	39	3 *(R)*	5	1
5	Lipase from *Candida cylindracea*	18	13	4 *(S)*	28	2

Reaction carried out using no enzyme gave no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]E_{calc} conversion and E were not determined as this requires *ee* > 1; [c]enantiomeric excess values were not determined as conversion was 0%; [d]enantiomeric excess values were not determined as full conversion was observed.

*Table 2.7 Hydrolysis of 2-Phenylpropyl pivalate **23** -Introduction of solvents and increasing temperature*



rac-**23** (*R*)-**23** (*S*)-**1**

Entry	Cosolvent	Conversion (%)		*ee*[a] (%)		E
		NMR	E_{calc}	ee_s	ee_p	
1	-[b]	21	19	20	88 *(S)*	20
2	Methyl *tert*-Butyl Ether	<1	-[c]	0	81 *(S)*	-[c]
3	2-Propanol	-[d]	4	4	91 *(S)*	22
4	*n*-Heptane	<1	-[c]	0	>99 *(S)*	-[c]
5	Acetonitrile	2	-[c]	0	>99 *(S)*	-[c]

Initial reactions were carried out at 30°C using the following enzymes, which gave conversion <10%: Hog pancreas lipase, Lipase from *Candida cylindracea*. The following enzymes gave no conversion: *Pseudomonas fluorescens* (immobilised), *Candida antarctica* lipase B (immobilised). [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]carried out at 30°C, all other reactions carried out at 50°C; [c]E_{calc} conversion and E were not determined as this requires *ee* > 1; [d]conversion by NMR could not be determined because of the presence of the solvent peak

2.4.5 Screening – isobutyrate

As a result of the high selectivity but poor conversion using the pivalate ester **23** the isobutyrate ester **24** was selected, as a compromise between the small acetate ester group,

which gave good conversion and poor selectivity, and the bulky pivalate ester group, which gave poor conversion but excellent selectivity (Figure 2.12).

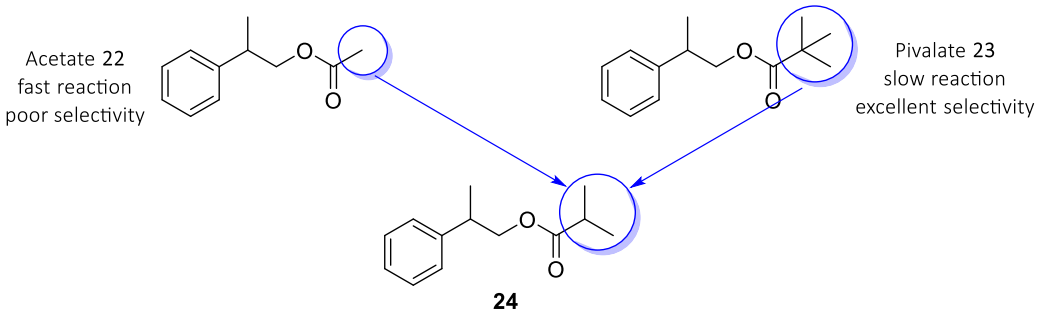
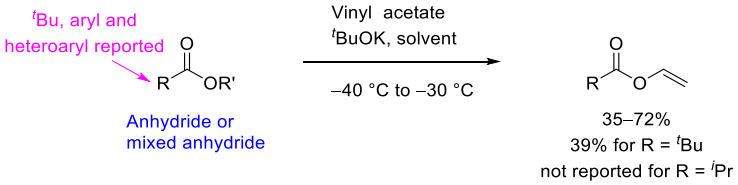


*Figure 2.12 2-Phenylpropyl Isobutyrate **24**: A compromise between ester groups*

Transesterification reactions were not carried out, partly because the vinyl ester was not commercially available. Possible synthetic routes are either low yielding, use complex or gaseous reagents or require high pressure, which would not be sufficient to access the amount of reagent required for screening reactions.[86] Scheme 2.24 shows a recently reported synthetic route to various aromatic vinyl esters through transvinylation of anhydrides or mixed anhydrides as reagents.[87]

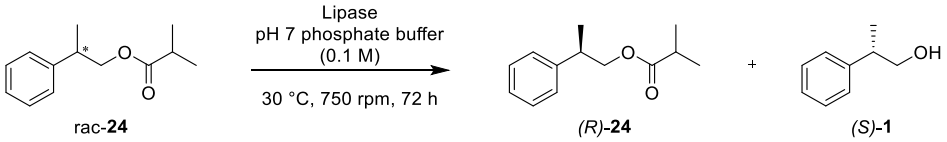


Scheme 2.24 Synthetic route to enol ethers

Candida antarctica Lipase B (immobilised) showed excellent enantioselectivity in hydrolysis leading to both the ester **24** and the alcohol **1** in good enantiopurity (

Table 2.8, Entry 2); the reaction was repeated several times and shows the reproducibility of the reaction. Amano PS Lipase also gave excellent enantioselectivity for the recovered ester, coupled with conversion >50% (Entry 4).

*Table 2.8 Hydrolysis of 2-phenylpropyl isobutyrate **24***



rac-**24** *(R)*-**24** *(S)*-**1**

Entry	Lipase	Conversion (%)		*ee*[a] *(%)*		E[88]
		NMR	E_{calc}	ee_s	ee_p	
1	Hog Pancreas lipase	17	15	11	60 *(S)*	4
2	*Candida antarctica* lipase B (immob)	51	53	91	82 *(S)*	32
		-[b]	45	73	90 *(S)*	41
		-[b]	47	79	90 *(S)*	48
		46	46	76	90 *(S)*	44
3	*Pseudomonas fluorescens* lipase (immob)	9	8	4	51 *(S)*	3
4	Amano PS lipase	64	67	81	40 *(S)*	5
		-[b]	63	81	47 *(S)*	6
		-[b]	51	61	58 *(S)*	7
5	Lipase from *Candida cylindracea*	100	-	-	-	-

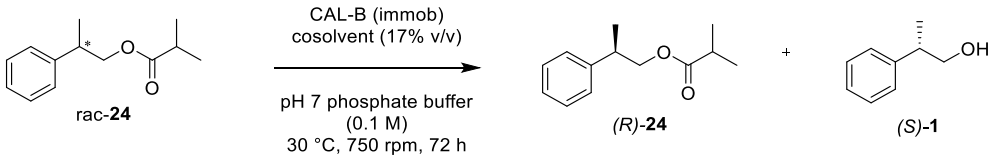
Reaction carried out with no enzyme present resulted in no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis using Chiralcel OBH, 0.5 ml/min, 99:1 hexane:IPA; [b]conversion was not determined by ^{1}H NMR.

The biocatalysed hydrolysis was subjected to an organic solvent screen (Table 2.9). E-values of up to 66 were achieved, with up to 95% *ee*. Water miscible and immiscible solvents were chosen and used as a 17% v/v mixture with the buffer. Only moderate conversions were attained using water miscible 2-propanol, acetone, and acetonitrile (Entries 3-5). The hydrocarbons pentane, hexane and heptane (entries 14, 8 and 6, respectively) gave similar conversions and enantioselectivities; interestingly, cyclohexane (entry 13) had much higher conversion than the acyclic *n*-hexane (Entry 8) (33% vs 12% conversion). Methyl *tert*-butyl ether (MTBE) and diisopropyl ether (Entries 7 and 9), two ether solvents having the same molecular formula, gave similar enantioselectivities (94% *ee* and 92% *ee*) but MTBE gave higher conversion (34% vs. 8%). Enantioselectivity towards the alcohol **1** was consistently high (87–95%).

Although *tert*-butanol is miscible with water, an interface was observed on addition of 17% *tert*-butanol to the reaction mixture; presumably, the buffer solution was sufficient to change the solubility of the solvent in the aqueous medium.

Recent literature has suggested that less polar solvents have a positive effect on the performance of *Candida antarctica* Lipase B, but no correlation was observed here; it should be noted, however, that the concentrations of solvents used were different in the literature report.[89]

*Table 2.9 Solvent screen of **24** with CAL-B (immob.)*

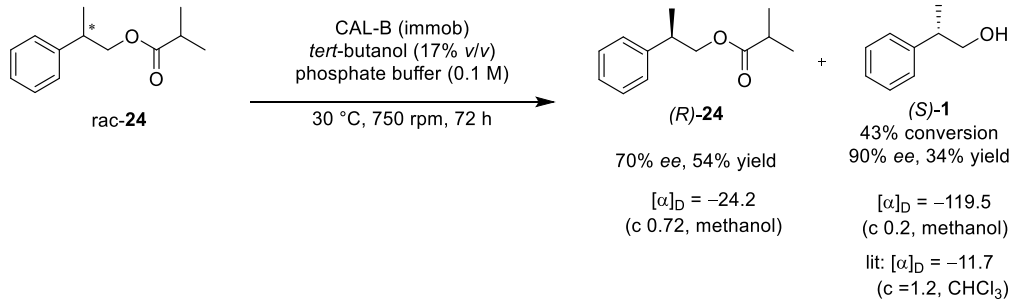


Entry	Cosolvent	Conversion[a] (%)	*ee*[b] (%)		E
			ee$_s$ *(R)*	*ee*$_p$ *(S)*	
1	-	46	76	90 *(S)*	44
2	1-Octanol	2	2	89 *(S)*	17
3	2-Propanol	21	24	93 *(S)*	33
4	Acetone[c]	33	47	95 *(S)*	60
5	Acetonitrile[c]	33	47	95 *(S)*	63
6	*n*-Heptane[c]	14	15	92 *(S)*	29
7	Methyl *tert*-butyl ether[c]	34	48	94 *(S)*	53
8	Hexane[c]	10	10	92 *(S)*	27
9	Diisopropyl ether	8	8	92 *(S)*	26
10	*tert*-Butanol	48	85	91 *(S)*	59
11	2-Methyltetrahydrofuran	14	15	95 *(S)*	41
12	Toluene	41	62	87 *(S)*	28
13	Cyclohexane	33	46	95 *(S)*	66
14	Pentane[c]	12	12	93 *(S)*	29

[a]E_{calc} conversion; [b]enantiomeric excess values were determined by chiral HPLC analysis; [c]HPLC grade solvent used.

2.4.6 Preparative scale synthesis of *(S)*-2-phenyl-1-propanol *(S)*-**1** and *(R)*-2-phenylpropyl isobutyrate *(R)*-**24**

Following on from this successful resolution screen, and as a conclusion to the study, the resolution of 2-phenyl-1-propanol **1** was carried out on a preparative scale. The solvent chosen for scale up was *tert*-butanol on the basis of both efficiency and selectivity (Table 2.9, Entry 10) furnishing 2-phenyl-1-propanol **1** in 34% isolated yield, and the ester **24** in 54% yield, after column chromatography, accompanied by 90% *ee* and 70% *ee*, respectively (Scheme 2.25). The literature rotation agrees with the measured rotation.[25]



*Scheme 2.25 Preparative scale synthesis of (S)-2-phenyl-1-propanol **1** and (R)-2-phenyl-1-propyl isobutyrate **24**.*

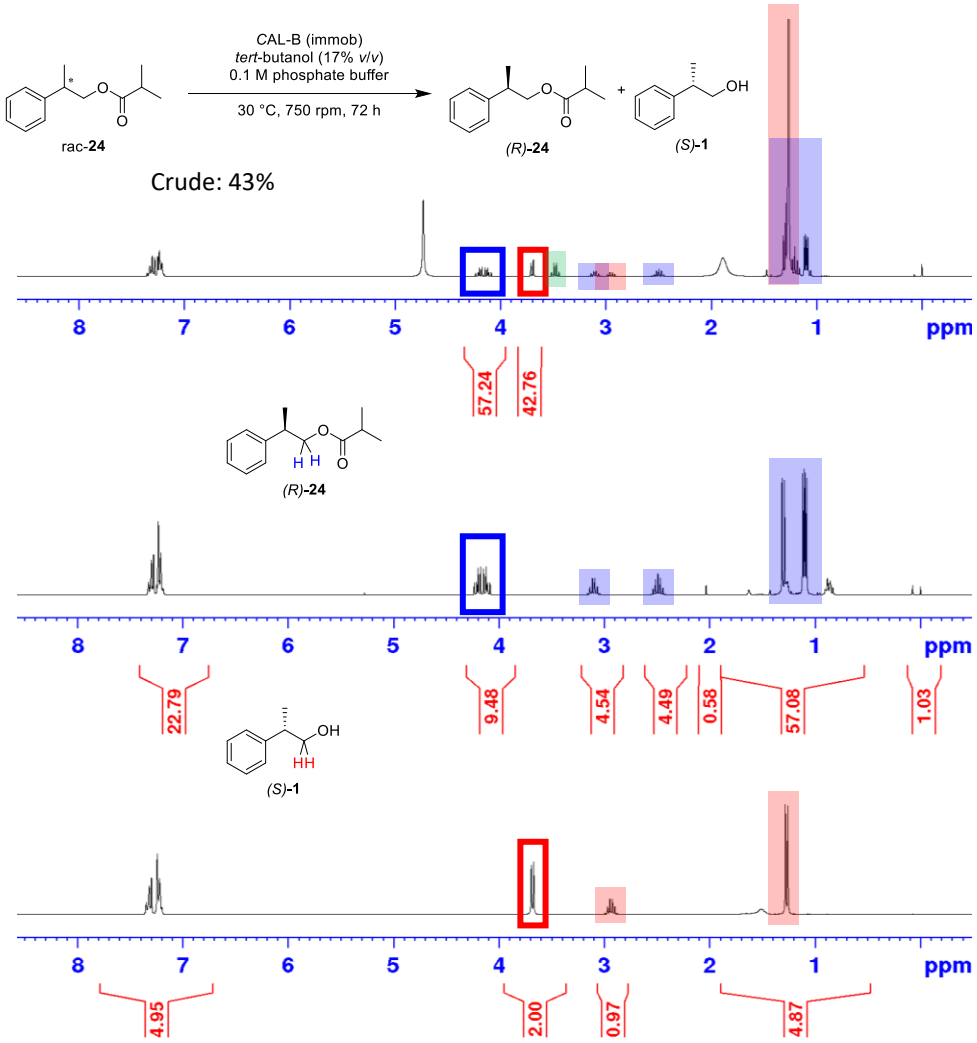


*Figure 2.13 ^{1}H NMR spectrum of the crude preparative scale reaction (top), the purified ester **24** (middle), and the purified alcohol **1** (bottom) ($CDCl_3$, 300 MHz)*

The isobutyric acid by-product was removed in the aqueous work-up, and was not detected in the crude ^{1}H NMR spectrum of the reaction mixture (expect singlet at 11.8 ppm, multiplet at 2.6 ppm and doublet at 1.22 ppm)[90] (Figure 2.13); only peaks corresponding to the alcohol product **1** and the ester substrate **24** were present. This is a clear advantage as this meant that only two components needed to be isolated from the crude mixture.

2.4.7 Determination of stereochemistry

The stereochemistry was determined by comparison to the reported optical rotation data and the HPLC trace of a sample of enantiopure (–)-*(S)*-2-phenyl-1-propanol *(S)*-**1** (Figure 2.14), purchased from Sigma Aldrich.[25] A sample of the racemic alcohol **1** was run on the HPLC, and a sample of enantiopure *(S)*-**1**. To confirm that the correct elution order had been identified a mixture of the racemic alcohol and the enantiopure alcohol was also run. The first peak was

enhanced relative to the racemic mixture, confirming that the *(S)*-enantiomer was eluted first. This was necessary, as it is known that the retention times of compounds can vary slightly, even under the same conditions.

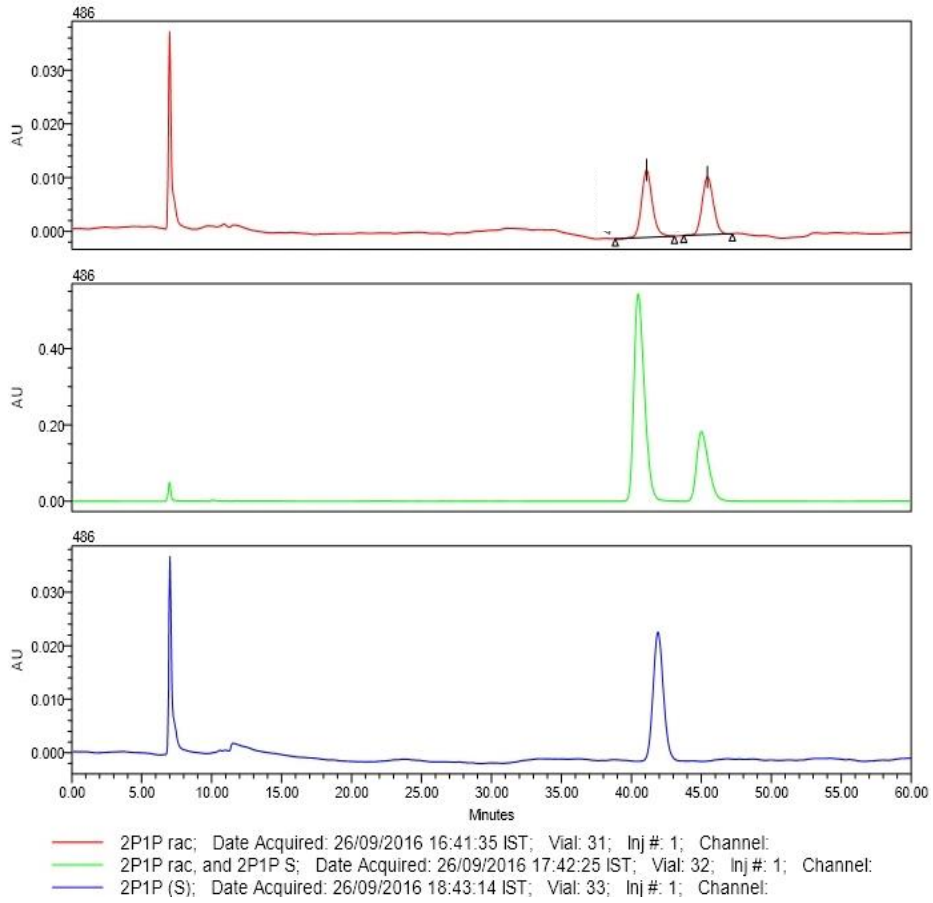


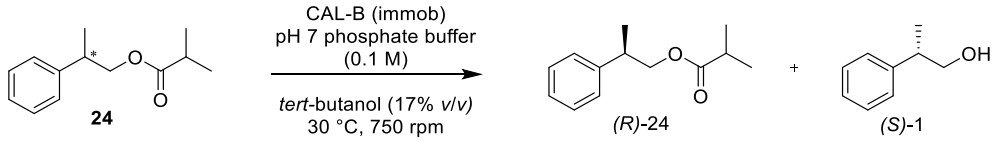
*Figure 2.14 HPLC trace showing racemic 2-phenyl-1-propanol **1** (red), (S)-**1** enriched material (green), and enantiopure (S)-**1** (blue) (Chiralcel OB-H, 0.5 mL/min, 0.5:99.5 2-propanol/hexane)*

2.4.7 Follow up – change lipase concentration

In order to ascertain if the reaction was a true resolution, the screen was repeated under the same conditions as the preparative scale resolution, for 24 h, 72 h and 120 h, except that the lipase concentration was doubled. The reactions attained 64% conversion, by ^{1}H NMR, after 24h, showing that the reaction proceeded faster with higher enzyme concentration. However, the reaction continued past 50% conversion. In this case, the ester selectivity was excellent, 97–99% *ee* as expected for conversion over 50%, with the alcohol selectivity decreasing with increasing conversion (Table 2.10). This shows us that ester **24** can be prepared with excellent enantioselectivity, when using a greater concentration of enzyme, in a much shorter time. However, the higher concentration of the lipase combined with a shorter time is unsuitable

for the preparation of the enantiopure alcohol **1** as the enantioselectivity is lower due to the greater extent of conversion (Table 2.10).

*Table 2.10 Hydrolysis of 2-phenylpropyl isobutyrate **24***



24 *(R)*-24 *(S)*-1

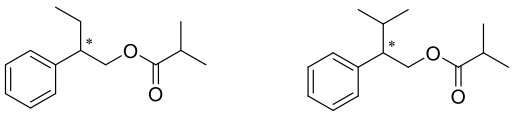
Entry	Time	Conversion (%)		*ee*[a] *(%)*		E[88]
		NMR	E_{calc}	ee_s	ee_p	
1	120	77	80	97	25	5.7
2	72	89	77	>99	29	7.6
3	24	64	56	>99	78	40

Reaction carried out with no enzyme present resulted in conversion <1% after 120 h. [a]Enantiomeric excess values were determined by chiral HPLC analysis.

Overall, these results indicate that by careful control of the reaction conditions and acyl group, the lipase-mediated hydrolysis can lead efficiently to highly enantioenriched 2-phenyl-1-propanol **1** and 2-phenylpropyl isobuyrate **24** in a synthetically useful process. The key to this was choosing the isobutyrate group as a compromise between the pivaloyl (highly selective, poorly efficient) and the acetyl (poorly selective and highly efficient) groups.

2.4.8 Follow up – substrate scope

Encouraged by the excellent results using 2-phenylpropyl isobutyrate **24**, the effect of increasing alkyl substituent size was briefly explored. Esters **25** and **26** (Figure 2.15) were subjected to hydrolysis using the optimised conditions for ester **24** [*Candida antarctica* lipase B (immobilised), 72 h, 750 rpm].



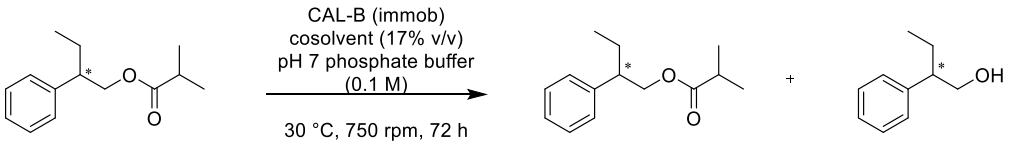
25 **26**

*Figure 2.15 Esters **25** and **26**, substrate scope.*

Ester **25** showed encouraging results for the initial screen using *Candida antarctica* lipase B (immobilised) (Table 2.11, Entry 1) and was subjected to a small solvent screen, using the solvents which gave the highest E-values for hydrolysis of 2-phenylpropyl isobutyrate **24**. Acetonitrile (Entry 4) showed the highest product enantioselectivity (86% *ee*), which was similar to the result for **24** (Table 2.9, entry 5). Previously, the highest reported %*ee* of **2** by

lipase-catalysed resolution (transesterification) was 69% *ee,* using vinyl acetate as the acyl source.[79] The more sterically demanding ester **26** was not hydrolysed either in neat buffer or with *tert*-butanol (17% *v*/*v*). The use of the ester having a *tert*-butyl substituent at the benzylic position was not explored.

*Table 2.11 Hydrolysis of 2-phenylpropyl isobutyrate **25** with CAL-B (immob)*



rac-**25** **25** **1**

Entry	Cosolvent	Conversion[a] (%)	*ee*[b] (%)		E
			ee_s	ee_p	
1	-	47	66	74	13
2	*tert*-Butanol	33	37	74	9.6
3	Acetone[c]	21	21	77	9.2
4	Acetonitrile[c]	24	28	86	17
5	Methyl *tert*-butyl ether[c]	19	19	82	12
6	Cyclohexane	18	17	82	12

[a]E_{calc} conversion; [b]enantiomeric excess values were determined by chiral HPLC analysis; [c]HPLC grade solvent used.

2.5 Project conclusion

A series of esters of 2-phenyl-1-propanol **1** was prepared, including two novel esters, **23** and **24**, and subjected to lipase-catalysed transformations. By manipulation of the reaction conditions, as well as modification of the substrate, the resolution of 2-phenyl-1-propanol **1** was optimised.

2-Phenyl-1-propanol **1** was resolved with excellent enantioselectivity, up to 96% *ee* and up to 48% conversion, using a commercially available lipase and a small, alkyl ester group, making the transformation selective and atom economical. E values of up to 63 were obtained; while this is lower than some reported values, it uses a much smaller ester group, making it much more atom economical, and easier to purify on scale up. A preparative scale resolution was also performed, giving 43% conversion after 72 hours, coupled with 34% yield of alcohol **1** with 88% *ee* and 54% yield of ester **24**, with 68% *ee*.

It was shown that the ester can also be resolved with excellent enantioselectivity (97–99% *ee*) by increasing the lipase concentration.

The influence of the size of the alkyl substituent at the chiral centre was also investigated. The ester **25**, bearing an ethyl group at the stereocentre, was subjected to lipase-catalysed hydrolysis, giving the alcohol **2** with up to 86% *ee*, the highest reported from a lipase catalysed transformation. On further increasing the size of the alkyl substituent to ^{i}Pr, the substrate **26** proved resistant to hydrolysis under the conditions used.

2.6 References

1. Bornscheuer, U.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*, 2006.
2. Wolfenden, R.; Snider, M. J. *Acc. Chem. Res.* 2001; *34*:938-45.
3. Ghanem, A. *Tetrahedron* 2007; *63*:1721-54.
4. Nakamura, K.; Kinoshita, M.; Ohno, A. *Tetrahedron* 1995; *51*:8799-808.
5. van Tilbeurgh, H.; Egloff, M.-P.; Martinez, C.; Rugani, N.; Verger, R.; Cambillau, C. *Nature* 1993; *362*:814-20.
6. Chen, C.-S.; Sih, C. J. *Angew. Chem. Int. Ed.* 1989; *28*:695-707.
7. Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. *Tetrahedron Lett.* 1998; *39*:7881-4.
8. Sakai, T.; Kawabata, I.; Kishimoto, T.; Ema, T.; Utaka, M. *J. Org. Chem.* 1997; *62*:4906-7.
9. Sabbani, S.; Hedenstrom, E.; Nordin, O. *J. Mol. Catal. B: Enzym.* 2006; *42*:1-9.
10. Jung, S.; Park, S. *ACS Catal.* 2017; *7*:438-42.
11. Itoh, T.; Mitsukura, K.; Kanphai, W.; Takagi, Y.; Kihara, H.; Tsukube, H. *J. Org. Chem.* 1997; *62*:9165-72.
12. Berglund, P.; Holmquist, M.; Hult, K.; Högberg, H.-E. *Biotechnol. Lett.* 1995; *17*:55-60.
13. Itoh, T.; Hiyama, Y.; Betchaku, A.; Tsukube, H. *Tetrahedron Lett.* 1993; *34*:2617-20.
14. Irimescu, R.; Saito, T.; Kato, K. *J. Mol. Catal. B: Enzym.* 2004; *27*:69-73.
15. Ardhaoui, M.; Falcimaigne, A.; Ognier, S.; Engasser, J. M.; Moussou, P.; Pauly, G.; Ghoul, M. *J. Biotechnol.* 2004; *110*:265-71.
16. Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *Tetrahedron: Asymmetry* 1996; *7*:625-8.
17. Goto, M.; Kawasaki, M.; Kometani, T. *J. Mol. Catal. B: Enzym.* 2000; *9*:245-50.
18. Kawasaki, M.; Goto, M.; Kawabata, S.; Kometani, T. *Tetrahedron: Asymmetry* 2001; *12*:585-96.
19. Prabhakar, S.; Vives, T.; Ferrieres, V.; Benvegnu, T.; Legentil, L.; Lemiegre, L. *Green Chem.* 2017; *19*:987-95.
20. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* 2000; *11*:1199-210.
21. Sabbani, S.; Hedenstrom, E.; Andersson, J. *Tetrahedron: Asymmetry* 2007; *18*:1712-20.
22. Nordin, O.; Nguyen, B.-V.; Vörde, C.; Hedenström, E.; Högberg, H.-E. *J. Chem. Soc., Perkin Trans. 1* 2000; *3*:367-76.
23. Isleyen, A.; Tanyeli, C.; Dogan, O. *Tetrahedron: Asymmetry* 2006; *17*:1561-7.
24. Meng, X.; Guo, L.; Xu, G.; Wu, J.-P.; Yang, L.-R. *J. Mol. Catal. B: Enzym.* 2014; *109*:109-15.
25. Serra, S. *Tetrahedron: Asymmetry* 2011; *22*:619-28.
26. Gotor-Fernández, V.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* 2006; *40*:111-20.
27. Basak, A.; Nag, A.; Bhattacharya, G.; Mandal, S.; Nag, S. *Tetrahedron: Asymmetry* 2000; *11*:2403-7.
28. Abate, A.; Brenna, E.; Fuganti, C.; Gatti, F. G.; Serra, S. *J. Mol. Catal. B: Enzym.* 2004; *32*:33-51.
29. Abate, A.; Brenna, E.; Fuganti, C.; Gatti, F. G.; Giovenzana, T.; Malpezzi, L.; Serra, S. *J. Org. Chem.* 2005; *70*:1281-90.
30. Elliott, G. A.; Purmalis, A.; Vandermeer, D. A.; Denlinger, R. H. *Toxicol. Pathol.* 1988; *16*:245-50.

31. Leitereg, T. J.; Guadagni, D. G.; Harris, J.; Mon, T. R.; Teranishi, R. *J. Agric. Food. Chem.* 1971; *19*:785-7.
32. Ammazzalorso, A.; Amoroso, R.; Bettoni, G.; De Filippis, B.; Fantacuzzi, M.; Giampietro, L.; Maccallini, C.; Tricca, M. L. *Chirality* 2008; *20*:115-8.
33. Berglund, P.; Vallikivi, I.; Fransson, L.; Dannacher, H.; Holmquist, M.; Martinelle, M.; Björkling, F.; Parve, O.; Hult, K. *Tetrahedron: Asymmetry* 1999; *10*:4191-202.
34. Moreno, J.; Sinisterra, J. *J. Mol. Cat. A: Chem.* 1995; *98*:171-84.
35. Moreno, J.; Samoza, A.; del Campo, C.; Llama, E. F.; Sinisterra, J. *J. Mol. Cat. A: Chem.* 1995; *95*:179-92.
36. Arroyo, M.; Sinisterra, J. V. *J. Org. Chem.* 1994; *59*:4410-7.
37. Hernáiz, M. J.; Sánchez-Montero, J. M.; Sinisterra, J. V. *J. Mol. Cat. A: Chem.* 1995; *96*:317-27.
38. Deasy, R. E.; Brossat, M.; Moody, T. S.; Maguire, A. R. *Tetrahedron: Asymmetry* 2011; *22*:47-61.
39. Deasy, R. E.; Moody, T. S.; Maguire, A. R. *Tetrahedron: Asymmetry* 2013; *24*:1480-7.
40. Foley, D. A.; O'Leary, P.; Buckley, N. R.; Lawrence, S. E.; Maguire, A. R. *Tetrahedron* 2013; *69*:1778-94.
41. Han, G.; Lewis, A.; Hruby, V. J. *Tetrahedron Lett.* 2001; *42*:4601-3.
42. Jaeger, K. E.; Reetz, M. T. *Trends Biotechnol.* 1998; *16*:396-403.
43. Avila, T. C.; Reginato, M. M.; Di Vitta, C.; Ducati, L. C.; Andrade, L. H.; Marzorati, L. *Tetrahedron Lett.* 2016; *57*:2152-7.
44. Sakai, T.; Hayashi, K.; Yano, F.; Takami, M.; Ino, M.; Korenaga, T.; Ema, T. *Bull. Chem. Soc. Jpn* 2003; *76*:1441-6.
45. Kawasaki, M.; Goto, M.; Kawabata, S.; Kodama, T.; Kometani, T. *Tetrahedron Lett.* 1999; *40*:5223-6.
46. Jaeger, K.-E.; Schneidinger, B.; Rosenau, F.; Werner, M.; Lang, D.; Dijkstra, B. W.; Schimossek, K.; Zonta, A.; Reetz, M. T. *J. Mol. Catal. B: Enzym.* 1997; *3*:3-12.
47. Chen, C. S.; Liu, Y. C. *J. Org. Chem.* 1991; *56*:1966-8.
48. Huang, Z.; Tan, Z.; Novak, T.; Zhu, G.; Negishi, E.-i. *Adv. Synth. Catal.* 2007; *349*:539-45.
49. Galletti, P.; Emer, E.; Gucciardo, G.; Quintavalla, A.; Pori, M.; Giacomini, D. *Org. Biomol. Chem.* 2010; *8*:4117-23.
50. Quaglia, D.; Pori, M.; Galletti, P.; Emer, E.; Paradisi, F.; Giacomini, D. *Process Biochem.* 2013; *48*:810-8.
51. Díaz-Rodríguez, A.; Ríos-Lombardía, N.; Sattler, J. H.; Lavandera, I.; Gotor-Fernández, V.; Kroutil, W.; Gotor, V. *Catal. Sci. Technol.* 2015; *5*:1443-6.
52. Kara, S.; Spickermann, D.; Schrittwieser, J. H.; Leggewie, C.; van Berkel, W. J. H.; Arends, I. W. C. E.; Hollmann, F. *Green Chem.* 2013; *15*:330.
53. Mazza, S.; Scopelliti, R.; Hu, X. *Organometallics* 2015; *34*:1538-45.
54. Gao, Y.; Jaenicke, S.; Chuah, G.-K. *Appl. Catal., A* 2014; *484*:51-8.
55. Liu, M.; Zhou, F.; Jia, Z.; Li, C.-J. *Org. Chem. Front.* 2014; *1*:161.
56. Li, Y.; Li, H.; Junge, H.; Beller, M. *Chem. Commun.* 2014; *50*:14991-4.
57. Souto, J. A.; Stockman, R. A.; Ley, S. V. *Org. Biomol. Chem.* 2015; *13*:3871-7.
58. Morimoto, T.; Fujii, T.; Miyoshi, K.; Makado, G.; Tanimoto, H.; Nishiyama, Y.; Kakiuchi, K. *Org. Biomol. Chem.* 2015; *13*:4632-6.
59. Zhuang, M.; Du, H. *Org. Biomol. Chem.* 2013; *11*:1460-2.
60. Guduguntla, S.; Fananas-Mastral, M.; Feringa, B. L. *J. Org. Chem.* 2013; *78*:8274-80.
61. Zhao, Y.; Weix, D. J. *J. Am. Chem. Soc.* 2014; *136*:48-51.
62. Ramage, R.; Blake, A. J.; Florence, M. R.; Gray, T.; Raphy, G.; Roach, P. L. *Tetrahedron* 1991; *47*:8001-24.

63. Scott, Natalie M.; Schareina, T.; Tok, O.; Kempe, R. *Eur. J. Inorg. Chem.* 2004; *2004*:3297-304.
64. Steib, A. K.; Thaler, T.; Komeyama, K.; Mayer, P.; Knochel, P. *Angew. Chem. Int. Ed.* 2011; *50*:3303-7.
65. Piller, F. M.; Metzger, A.; Schade, M. A.; Haag, B. A.; Gavryushin, A.; Knochel, P. *Chem. Eur. J.* 2009; *15*:7192-202.
66. Campos, J.; Espada, M. F.; Lopez-Serrano, J.; Carmona, E. *Inorg. Chem.* 2013; *52*:6694-704.
67. Padial, J. S.; de Gelder, R.; Fonseca Guerra, C.; Bickelhaupt, F. M.; Mecinovic, J. *Chem. Eur. J.* 2014; *20*:6268-71.
68. Nakamura, M.; Oki, M. *Bull. Chem. Soc. Jpn* 1980; *53*:2977-80.
69. Francisco, M. A.; Kurs, A.; Katritzky, A. R.; Rasala, D. *J. Org. Chem.* 1988; *53*:4821-6.
70. Rose, C. B.; Taylor, S. K. *J. Org. Chem.* 1974; *39*:578-81.
71. Ciaccio, J. A.; Volpi, S.; Clarke, R. *J. Chem. Edu.* 1996; *73*:1196-8.
72. Rioz-Martinez, A.; de Gonzalo, G.; Torres Pazmino, D. E.; Fraaije, M. W.; Gotor, V. *J. Org. Chem.* 2010; *75*:2073-6.
73. Karamé, I.; Tommasino, M. L.; Lemaire, M. *Tetrahedron Lett.* 2003; *44*:7687-9.
74. Robinson, M. W. C.; Pillinger, K. S.; Mabbett, I.; Timms, D. A.; Graham, A. E. *Tetrahedron* 2010; *66*:8377-82.
75. Umeda, R.; Muraki, M.; Nakamura, Y.; Tanaka, T.; Kamiguchi, K.; Nishiyama, Y. *Tetrahedron Lett.* 2017; *58*:2393-5.
76. Adam, W.; Bosio, S. G.; Turro, N. J.; Wolff, B. T. *J. Org. Chem.* 2004; *69*:1704-15.
77. Corberan, R.; Mszar, N. W.; Hoveyda, A. H. *Angew. Chem. Int. Ed.* 2011; *50*:7079-82.
78. Foley, A. M.; Gavin, D. P.; Joniec, I.; Maguire, A. R. *Tetrahedron: Asymmetry* 2017; *28*:1144-53.
79. Sih, J. C.; Gu, R. L. *Tetrahedron: Asymmetry* 1995; *6*:357-60.
80. Kovács, B.; Megyesi, R.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* 2017; *28*:1829-33.
81. Strohalm, H.; Dold, S.; Pendzialek, K.; Weiher, M.; Engel, K. H. *J. Agric. Food. Chem.* 2010; *58*:6328-33.
82. Takemura, T.; Saito, K.; Nakazawa, S.; Mori, N. *Tetrahedron Lett.* 1992; *33*:6335-8.
83. Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* 1996; *7*:3285-94.
84. Naemura, K.; Fukuda, R.; Konishi, M.; Hirose, K.; Tobe, Y. *J Chem Soc Perk T 1* 1994; *10*:1253-6.
85. Bianchi, D.; Battistel, E.; Bosetti, A.; Cesti, P.; Fekete, Z. *Tetrahedron: Asymmetry* 1993; *4*:777-82.
86. Mondal, M. A. S.; Van Der Meer, R.; German, A. L.; Heikens, D. *Tetrahedron* 1974; *30*:4205-7.
87. Kaliyaperumal Appaye, S.; Pandurang Nikumbh, S.; Reddy Govindapur, R.; Banerjee, S.; Bhalerao, D. S.; SyamKumar, U. K. *Helvetica Chimica Acta* 2014; *97*:1115-22.
88. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* 1982; *104*:7294-9.
89. Dutta Banik, S.; Nordblad, M.; Woodley, J. M.; Peters, G. H. *ACS Catal.* 2016; *6*:6350-61.
90. Dintzner, M. R.; Mondjinou, Y. A.; Pileggi, D. J. *Tetrahedron Lett.* 2010; *51*:826-7.

Chapter 3

Resolution of 6-methylchroman-2-ol: towards the hydrolase-mediated resolution of the hemiacetal in 2-chromanols

Contents

Overview

Hydrolase-catalysed kinetic resolution is a powerful tool for the resolution of chiral compounds, although it suffers from a maximum theoretical yield of 50%. Combining the kinetic resolution with a racemisation step, either through use of an external catalyst or through selection of a substrate which will racemise under the reaction conditions, furnishes a dynamic system, which is synthetically more powerful.

Hydrolase-catalysed dynamic kinetic resolutions of chroman-2-ol **50** and 6-methyl chroman-2-ol **51** was effected with up to 88% conversion and 92% *ee* through the use of organic solvents. Extension to the resolution of the tolterodine precursor **52** proved more challenging. The presence of the remote phenyl substituent had a significant impact on the resolution and it was not possible to achieve high enantioselectivity together with efficient conversion from the focused panel of enzymes screened.

3.1 Introduction

Coumarins, or benzo-α-pyrones, are a class of molecules made up of a benzene ring fused to a pyrone ring. They are present in many naturally occurring compounds and their derivatives have been used in pharmaceuticals such as anticoagulants,[1] and anti-cancer agents.[2] The numbering system is shown in Figure 3.1.

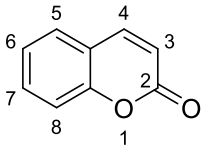
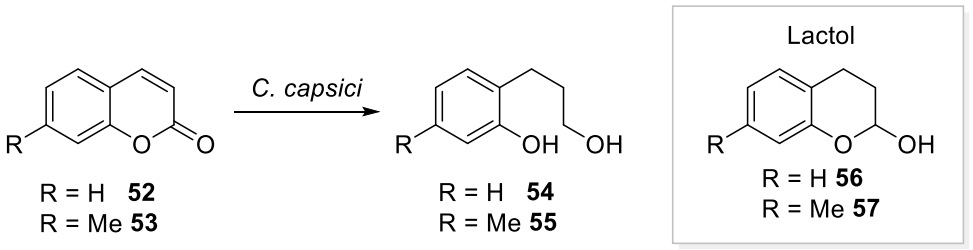
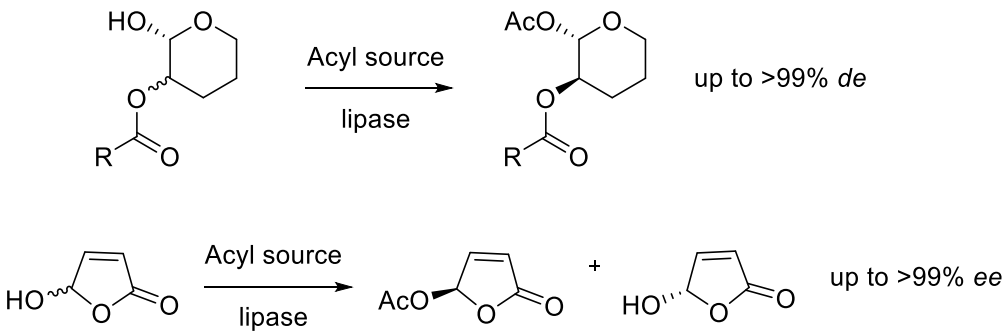


Figure 3.1 Numbering of Coumarins

Coumarins **52** and **53** have been used as substrates for enzymatic reduction giving the primary alcohols **54** and **55**, as a result of the reduction of the intermediate aldehyde (Scheme 3.1).[3] However, there are no reports of enzymatic resolution of the anomeric centre of the corresponding lactol compounds **56** and **57** so initially a model substrate was used in order to show that this centre could be resolved.[4] Previous reports of resolution of anomeric centres use monocyclic sugars as substrates (Scheme 3.2).[5-11]



*Scheme 3.1 Enzymatic reduction of coumarins **52** and **53** by C. capsici; corresponding lactols **56** and **57**.*



Scheme 3.2 Examples of lipase-catalysed resolution of the anomeric centres of sugar molecules

The lactol moiety is present in potential intermediates in the synthesis of muscarinic antagonists, tolterodine **58** and fesoterodine **59** (Figure 3.2). It is envisioned that the lactol alcohol could be dynamically resolved using lipase-catalysed transesterification, taking advantage of the dynamic ring-opening and ring-closing, which happens in solution. Once it has been shown that the dynamic resolution is possible, using the model lactol **50** and **51**, work can then follow looking at the kinetic resolution of the remote stereocentre in the Tolterodine and Fesoterodine intermediates **60** and **61**.

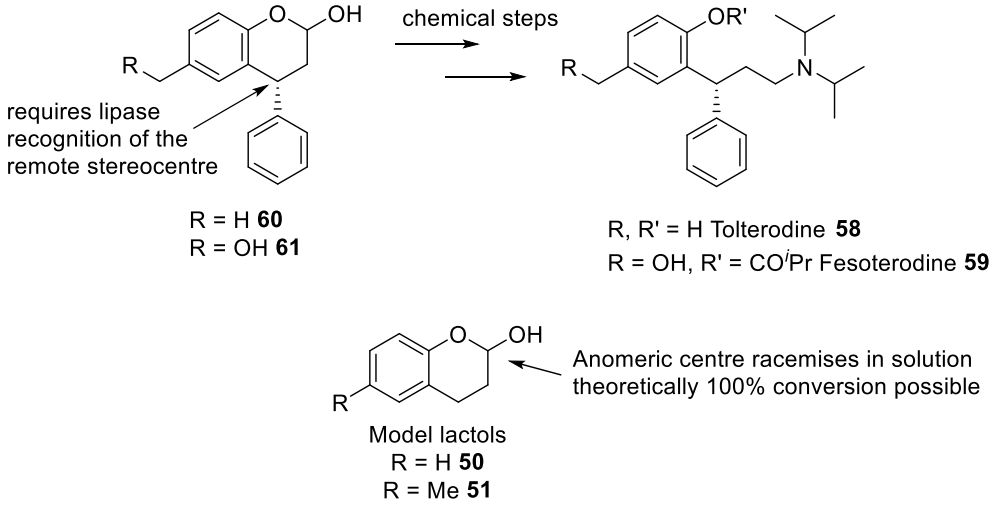
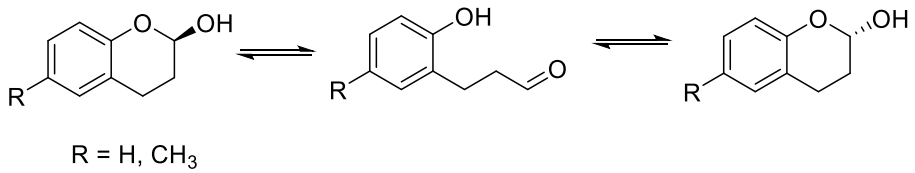


*Figure 3.2 Intermediate lactols **60** & **61**, and the model lactol, 6-methylchroman-2-ol **51***



Scheme 3.3 Dynamic equilibrium of the lactols with the ring-opened hydroxyaldehyde

The main goal here has been the synthesis and resolution of the title compound **51** as a preliminary step in the investigation of the resolution of intermediates in the synthesis of the tolterodine **58** and fesoterodine **59** (Figure 3.2).

The lactols exists in a dynamic equilibrium with the corresponding acyclic hydroxyaldehydes (Scheme 3.3). This is important, because it theoretically allows for complete conversion, via a dynamic kinetic resolution. The resolution of the lactol intermediates in the synthesis of tolterodine **58** and fesoterodine **59** will require, in addition, recognition of a stereocentre remote from the hemiacetal reacting site. Lipases have been shown to recognize remote stereocentres in compounds with an OH group (e.g. 3-arylalkanols).[12,13]

This preliminary research was subsequently extended within the team to the resolution of the API intermediates **60** and **61** (Figure 3.3).[4] The use of enzymes to resolve these intermediates represents a move towards a greener synthesis of these compounds.

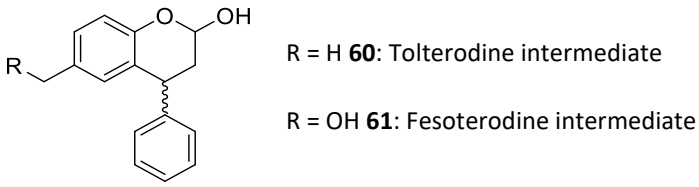
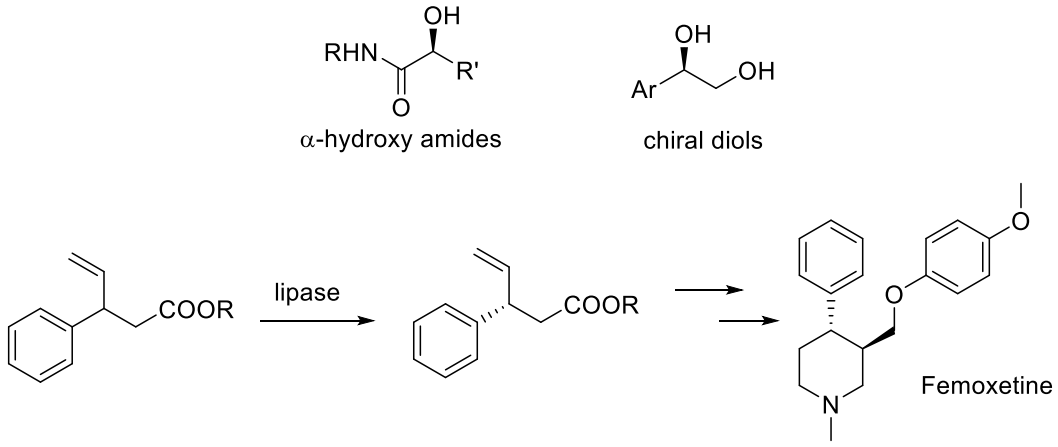


*Figure 3.3 Target compounds: intermediate in the synthesis of tolterodine **58** and fesoterodine **59***

There have been many reports of lipase mediated kinetic resolution of key synthetic intermediates, such as α-hydroxyamides,[14] chiral diols,[15,16] and other drug products and intermediates (Scheme 3.4).[17-19]

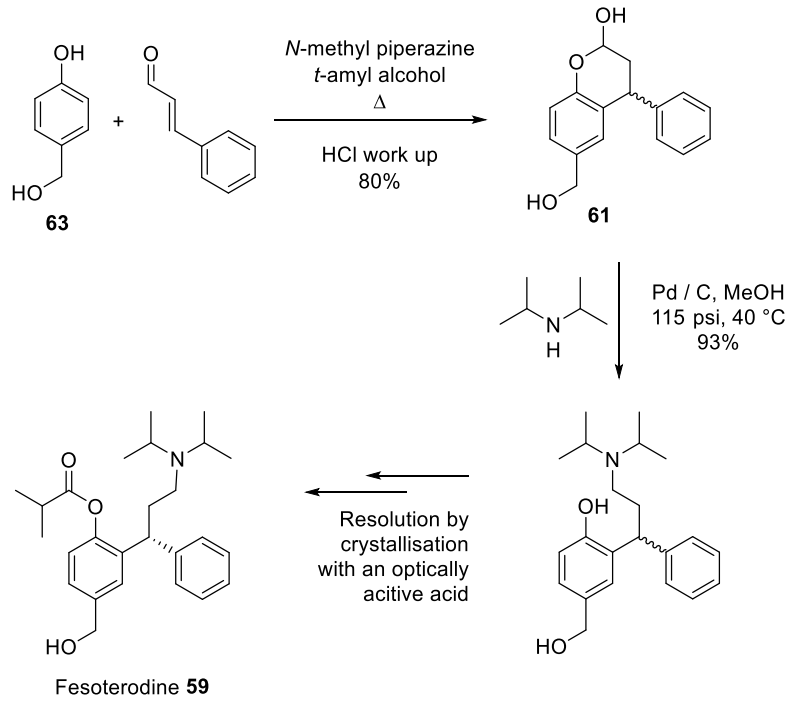


Scheme 3.4 Some key synthetic intermediates which have been resolved by lipases

3.1.1 Routes to tolterodine **58** & fesoterodine **59**

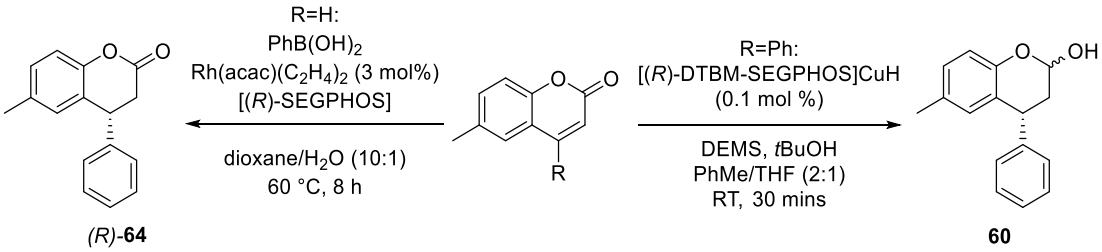
There is no shortage of routes to coumarins, dihydrocoumarins and lactols, each with advantages and disadvantages, including substrate scope and positions of substitution.[20-29]

The current route to fesoterodine **59** is by an amine-catalysed Friedel–Crafts alkylation of the substituted phenol **63** with cinnamaldehyde, followed by a reductive amination of the resulting lactol **61**, and resolution by crystallisation with an enantiopure acid in order to resolve the mixture of enantiomers (Scheme 3.5).[30] Enantiopure compounds are expensive, so the use of cheaply produced enantioenriched material potentially represents a more economical route.



*Scheme 3.5 Synthesis of fesoterodine **59***

The route to tolterodine **58** has been optimized more extensively. First patented in 1998, the original synthetic route relies on the use of a diastereomeric salt to separate the two enantiomers of tolterodine **58** and obtain the active enantiomer, (*R*)-**58**.[31,32] Early work used chiral auxiliaries, such as *N*-oxazolidinones[33] and enol ethers,[34] in conjunction with cinnamic acid **20**, and *o*-quinone methides, respectively. This allowed introduction of the stereocentre early in the synthesis and gave excellent enantioselectivity (>99%). Later, the use of SEGPHOS ligands was reported, with excellent results (Scheme 3.6). The reactions used either coumarins unsubstituted at the 4-position, with the ruthenium mediated stereoselective 1,4-addition of aryl boronic acids to give the lactone **64**, or had the substituent present already and used a copper hydride catalyst to stereoselectively reduce the double bond.[35,36] The latter route had the advantage of furnishing the lactol product **60**, eliminating the need for a separate reduction. The use of alternative ligands has also been reported for the synthesis of *(R)*-**64** including ligands where an early synthetic step in the synthesis of the ligand includes a lipase-catalysed resolution.[37]



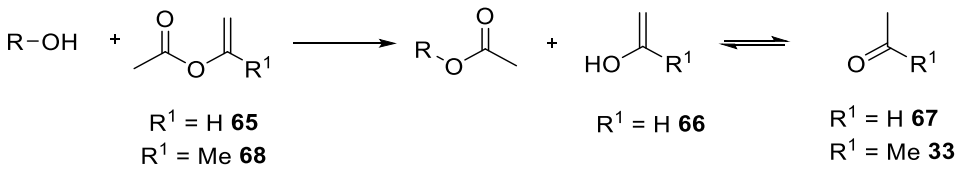
*Scheme 3.6 Enantioselective synthesis of Tolterodine intermediates **64** & **60** using Rh[35] & Cu[36] catalysis*

3.1.2 Enzymatic resolution

Although enzymatic resolution of the hemiacetal moiety has been reported previously,[5-11] hydrolase-mediated resolution of aromatic fused hemiacetals has not previously been reported. A model substrate (dihydrochroman-2-ol **51**) was previously synthesized and screened within our group achieving up to 95% *ee* through hydrolase-mediated acylation.[4] This will be discussed with Table 3.3.

3.1.3 Acylating agents

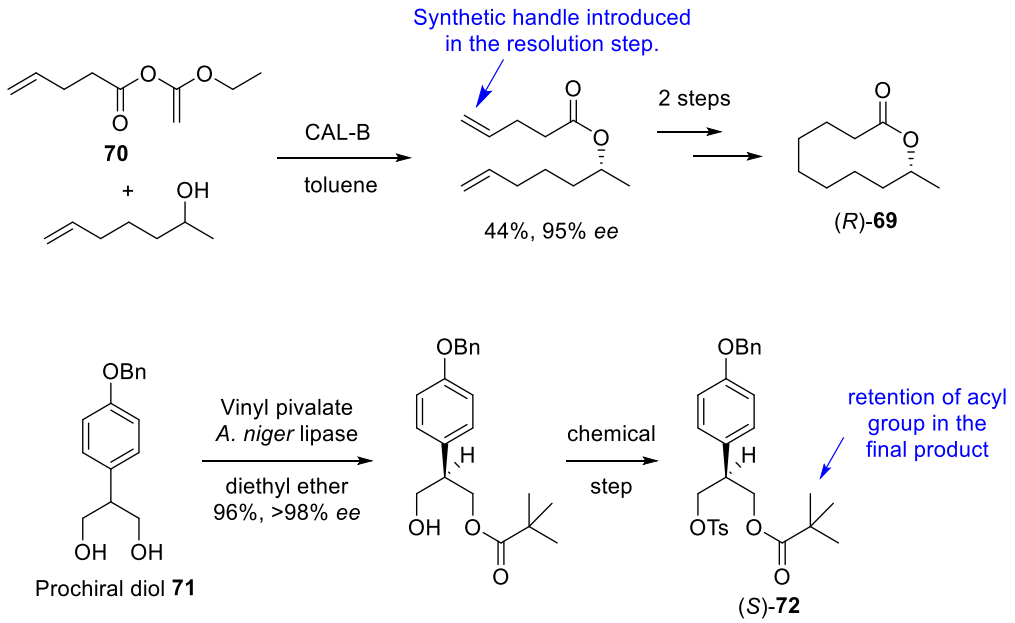
The choice of acylating agent in these reactions is important from a synthetic perspective, because one challenge with enzymatic acylation is the reversibility. The use of an acylating agent such as vinyl acetate **65** can overcome this challenge because the enol by-product **66** is unstable and tautomerises to acetaldehyde **67**, rendering the reaction irreversible (Scheme 3.7).[38] The use of vinyl acetate also has the advantage that there is only a small amount of non-selective background acylation. Isopropenyl acetate **68**, which is a stronger acylating agent than **65**, was also used during this study.[39] Much stronger acylating agents, such as acetic anhydride, would be expected to give higher conversion but lower selectivity. Succinic anhydride has been used in conjunction with lipases giving good selectivity in the resolution of alkyl alcohols.[40,41] The use of 1-ethoxyvinyl furoate has been reported for the resolution of 1,2- and 1,3- diols.[35]



*Scheme 3.7 Acetylation using **68** or **65** and generation of **33** or **67** as a side product*

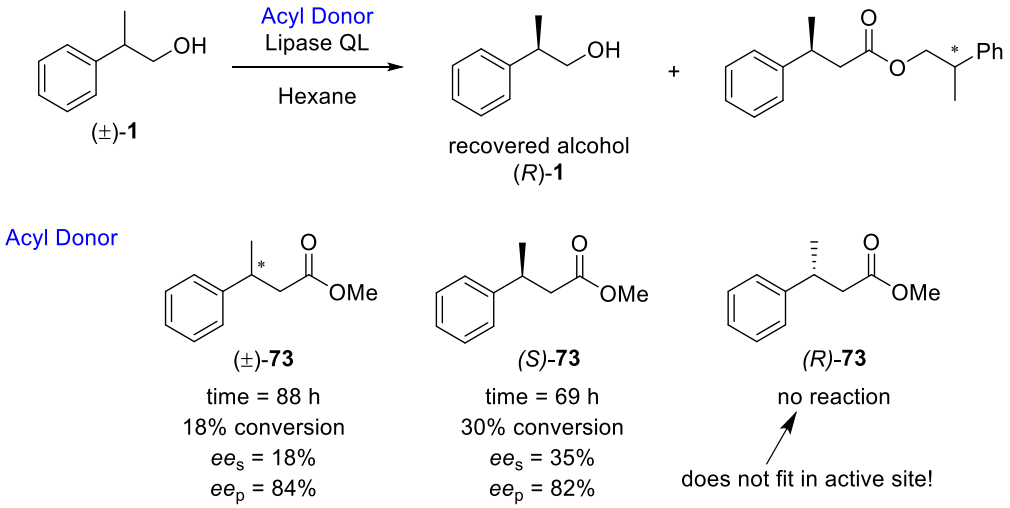
The use of longer-chain acylating agents has been reported to improve selectivity and conversion.[42-44] This is attributed to interaction of the alkyl chain with a hydrophobic channel near the binding site.[44]

Chênevert et al. investigated the resolution of 1-phenylethanol using a series of different acylating agents in order to assess the viability.[13] The ability to use different acylating agents could expand the utility of these reactions, not only allowing the resolution of the compounds but also the introduction of functional groups, which would be especially useful in the synthesis of natural products. Scheme 3.8 shows the synthesis of macrolide natural product (*R*)-phoracantholide J (*R*)-**69**, where the use of a carefully chosen acyl source **70** allowed the introduction of a synthetic handle. A prochiral diol **71**, with a remote stereocentre, was resolved using vinyl pivalate, allowing the simultaneous resolution of the compound and introduction of a pivalate group, which was retained in the final compound (*S*)-**72**.



*Scheme 3.8 Retention of acyl group used in resolution in the synthesis of protein kinase ligand (S)-**72** (bottom)*

Hirose et al. found that when a chiral acyl donor, such as **73**, was used in the resolution of 2-phenyl-1-propanol **1**, one enantiomer was inactive (Scheme 3.9).[45] When the inactive enantiomer (*R*)-**73** was screened, it furnished no acylated product. Using the active enantiomer (*S*)-**73** did not improve selectivity over the racemic mixture, but it did give greater conversion, which is attributed to less competition within the binding site.



*Scheme 3.9 Use of chiral acyl donors to resolve 2-phenyl-1-propanol **1**; one enantiomer of acyl donor **73** was inactive*

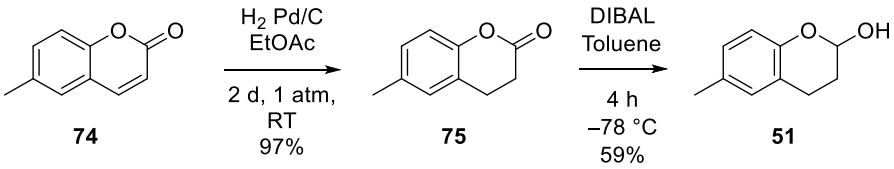
3.2 Objectives

The specific objectives of this project are:

- To prepare the model lactol, 6-methylchroman-2-ol **51** and prepare the acyl derivative **62** and develop analytical conditions to determine enantiomeric excess of the acyl derivatives.
- Use the model lactol **51** as a substrate for lipase-mediated transesterification screens, using a targeted panel of lipases.
- Identify lead enzymes and carry out a solvent screen.
- To conduct a preparative scale resolution of the compound and identify the enantiomer formed.

3.3 Synthesis of the model lactol substrate **51**

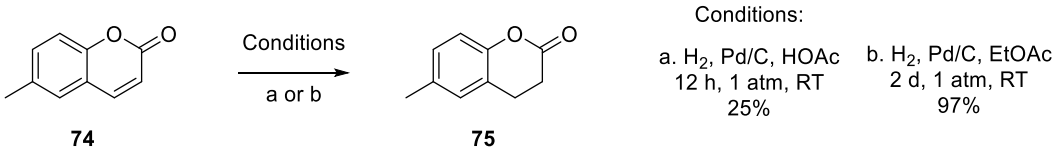
The lactol substrate **51** was synthesized using a two-step method, first reducing the alkene bond of **74** with H_2, Pd/C, followed by reduction of the carbonyl double bond of **75** using DIBAL.[46,47] Commercially available 6-methyl coumarin **74** was used as starting material (Scheme 3.10).



*Scheme 3.10 Synthesis of 6-methyl lactol **51***

3.3.1 Reduction of the alkene moiety

The first attempt at reduction of the alkene used acetic acid as a solvent and was complete in 12 h (Scheme 3.11). Unfortunately the yield was much lower than expected (25% vs >95% reported).[46] The lower yield may be caused by base-catalysed hydrolysis of the lactone, which would then remain in the aqueous layer.



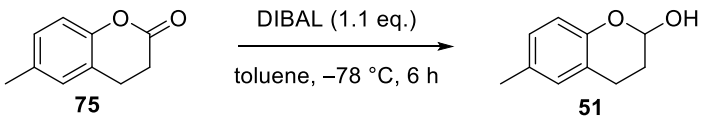
Scheme 3.11 Reduction of the alkene

The reduction of the alkene using ethyl acetate as the solvent, proceeded with 97% yield, gave a very clean product after drying and required no purification, albeit 48 h was required for complete consumption of the starting material.[48,47] Despite the longer reaction time, this method was found to be high yielding and gave a pure product **75** (Scheme 3.11). The same reaction was reported using a Parr hydrogenator at 57 psi, giving the product after only 3 h but it was not attempted in this investigation.[49]

3.3.2 Reduction of the lactone **75**

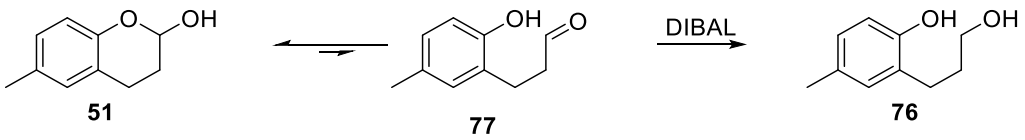
The reduction of the lactone **75** was performed under anhydrous conditions, at low temperature, with 1.1 eq. of DIBAL, as a 1M solution in toluene, using freshly distilled toluene

as solvent (Scheme 3.12). Several different sets of conditions were used to optimize the yield (Table 3.1).



*Scheme 3.12 Reduction of lactone **75** to lactol **51***

For the first attempt, TLC showed that, even after 4 h, starting material still remained. In this case, an extra 0.55 equivalents of DIBAL were added and stirred at low temperature until the starting material had disappeared. In this case, however, two products were recovered after column chromatography in a ratio of ~2:1. The major product was the desired lactol product **51**, and the minor was identified as the over-reduction product, diol **76** (Scheme 3.13).



*Scheme 3.13 Reduction of the lactol **51** to the diol **76** via the aldehyde intermediate*

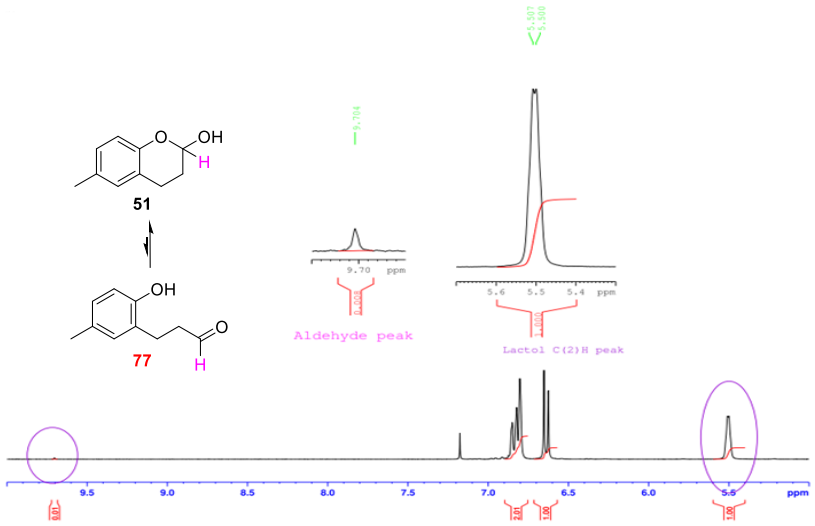


Figure 3.4 ^{1}H NMR showing aldehyde peak and lactol ^{1}H peak, expansions are not to scale ($CDCl_3$, 300 MHz)

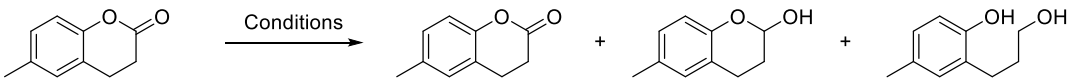
The lactol product exists in an equilibrium with the ring opened aldehyde **77**. The aldehyde is present in a very small amount (<1%, in $CDCl_3$) and appears in the ^{1}H NMR at 9.8 ppm (Figure 3.4). In the case above, the aldehyde form of the lactol **51** is further reduced to the corresponding alcohol **76** (Table 3.1, Entry 1). The data obtained for the diol **51** matched previously reported data for the compound.[3]

Further experiments required up to 6 h for the reaction to occur, but it was decided that after this time, that the reaction would be worked up, allowing the recovery of starting material which could be reused, along with the product, due to the challenge of maintaining the temperature for extended times overnight. The overall conversion after 6 h was calculated from the ^{1}H NMR spectrum and was ~80% (Table 3.1, Entry 2).

Purification of **51** was generally difficult, requiring several columns to effectively separate the starting material **75** and product **51**. The unreacted dihydrocoumarin **75** eluted off the column first, then fractions containing a mixture of the unreacted dihydrocoumarin **75** and the product **51** (generally composed of 70–80% product), followed by the product. Immediately after concentration, the product was analysed and dried under vacuum overnight.

The addition of molecular sieves, to remove water during the reaction, gave much better conversion, resulting in no unreacted starting material **75**, after 6 h reaction (Table 3.1, Entry 3). The material obtained from this reaction was obtained in high purity, but chromatography was undertaken prior to enzymatic reactions as the material was required in very high purity. The synthesis of lactol **51** has been previously reported.[49]

*Table 3.1 Synthesis of lactol **51***



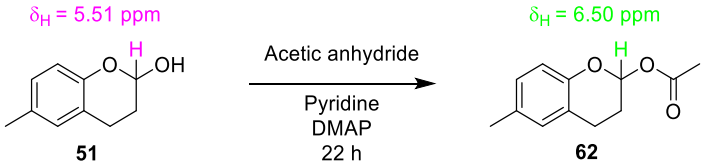
75 **75** **51** **76**

Entry	Conditions	% 75[a]	% 51[a]	% 76[a]
1	DIBAL (1.1 eq.), toluene, −78°C, 4 h DIBAL (0.55 eq.), 2 h	0	57	43
2	DIBAL (1.1 eq.), toluene, −78°C, 6 h	20	80	0
3	DIBAL (1,1 eq.), Toluene, −78°C, 6 h 4Å molecular sieves	0	100	0

[a] % composition determined by ^{1}H NMR of crude reaction mixture.

3.3.3 Acylated lactol reference standard

In order to develop analytical methods for the reactions, it was necessary to prepare the acylated lactol **62** (Scheme 3.14). The acylation of the lactol **51** was carried out using a general acylation method previously used in our group employing acetic anhydride as the acylating agent, distilled DCM as solvent, *N*,*N*-dimethylaminopyridine (DMAP) as nucleophilic catalyst (5 mol %), and pyridine as a base (4.5 eq.).[50] The reaction proceeded at room temperature, and gave full conversion on stirring overnight. This was evident by the disappearance of the 1H signal at 5.58 ppm, and the appearance of a 1H signal at 6.50 ppm. Aqueous copper sulfate solution was employed during the work-up to remove pyridine, and any excess, which was not removed by the work-up, was removed by azeotrope with heptane. Although the ^{1}H NMR spectrum showed complete acylation to the pure acetate **62**, the product was subjected to column chromatography as it was required in very high analytical purity. This compound is novel and was fully characterized in the course of this investigation.

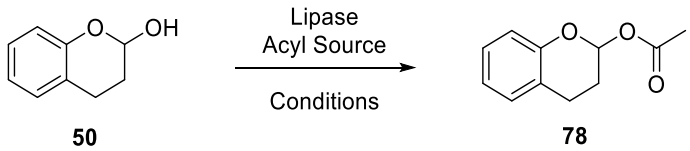


*Scheme 3.14 Preparation of acylated lactol **62***

3.4 Resolution of lactol **51**

3.4.1 Enzymatic screen of lactol substrate – background on related substrate

Previously, up to 95% *ee* was achieved for the unsubstituted lactol **50**, using neat vinyl acetate **65** (100 eq.) (Scheme 3.15).[4] However, it was observed that >70% *ee* was always coupled with low conversion, and that there was reduced enantioselectivity when the conversions were higher. Most enzymes which were screened furnished no acetylated product **78**. An investigation into the effect of organic solvents on the conversion and selectivity had also been conducted on this compound in which up to 91% *ee* was obtained, albeit with a low conversion (13%). Within this study, conversions of up to 81% were achieved, coupled with 56% *ee*. In general, it was observed that use of non-polar solvents gave better conversion and enantioselectivity. It should be noted that only 4.2 equivalents of the acylating agent were used in the solvent screen for this compound, but a higher loading of vinyl acetate was used in the screens using 6-methylchromanol **51** as substrate.



50 **78**

*Scheme 3.15 Resolution of unsubstituted lactol **50***

3.4.2 Analysis of reactions

The reaction completion (conversion) was determined by ^{1}H NMR. The peaks for the C(2) protons of the starting material **51** and the desired (acylated) product **62** were sufficiently separated on the ^{1}H NMR spectrum (Figure 3.5) and were not overlapping with any other peaks. The enantiomeric excess was determined by chiral HPLC and the HPLC conditions are listed in **Appendix I**. The reactions were worked up by simply filtering the reaction solution through Celite® and removing the solvent by rotary evaporation. The ^{1}H NMR analysis was carried out on the crude material, which only contained the unreacted lactol **51** and the acylated product **62**.

The results of the initial screens are shown in Table 3.2, and the results of the solvent screen are shown in Table 3.5.

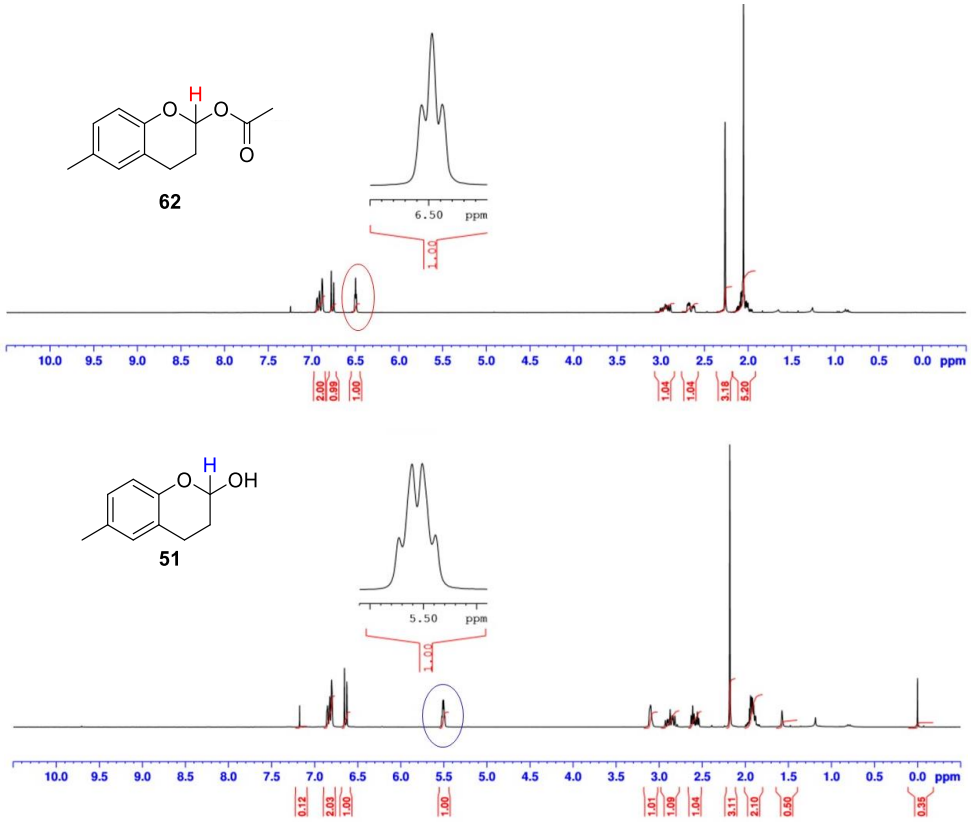


*Figure 3.5 ^{1}H NMR spectra for the lactol **51** and acetate **62** ($CDCl_3$, 300 MHz)*

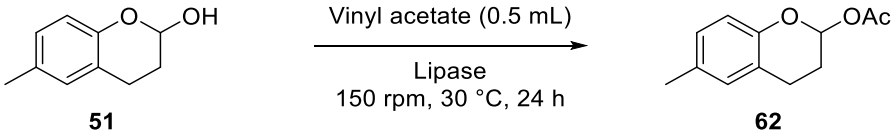
3.4.3 Initial 24-hour screen

The initial 24 h screens were carried out using vinyl acetate as solvent and acylating agent (Table 3.1). The lactol substrate **51** was dried prior to the reactions, and the vinyl acetate, which was stored in the fridge, was allowed to warm to room temperature before opening to minimize the ingress of water. The presence of water in these reactions could lead to the hydrolysis of the product, although strictly anhydrous conditions were not used. Control reactions were carried out, and, in the absence of an enzyme, there was no background acylation evident.

Several lead enzymes were identified for the solvent screen. Enzymes were chosen mainly on the basis of activity, but those with poor selectivity were disregarded, as high selectivity is important in this investigation. Lipase E from *Alcaligenes sp.* gave the highest conversion, coupled with moderate enantioselectivity (Table 3.1, Entry 1). Lipase from *Candida cylindracea*

and lipases A and B from *Candida rugosa*[1] performed similarly, with a slightly lower conversion, with moderate selectivity. The lipase from *Thermomyces lanuginosus* gave excellent selectivity (97% *ee*), albeit coupled with lower conversion than the other lead enzymes (Entry 7).

Table 3.2 Initial lactol screening results using vinyl acetate as acylating agent



51 **62**

Entry	Hydrolase Source	Conversion (%)[a]	*ee* (%)[b]
1	Lipase E from *Alcaligenes sp.*	30	70
2	Lipase from *Candida cylindracea*	28	65
3	Lipase A from *Candida rugosa*	26	64
4	*Candida antarctica* Lipase A	26	8
5	Lipase B from *Candida rugosa*[c]	20	59
6	Lipase from *Pseudomonas stutzeri*	19	33
7	Lipase from *Thermomyces lanuginosus*	12	97
8	Lipase B from *Candida rugosa*[c]	7	79
9	Lipase D from *Alcaligenes sp.*	6	66
10	Lipase F from *Alcaligenes sp.*	5	39
11	Lipase B from *Burkholderia cepacia*	5	74
12	Lipase from *Pseudomonas fluorescens* (immob)	5	83
13	Lipase from *Candida antarctica*	4	93
14	Lipase from *Pseudomonas cepacia*	3	80
15	Lipase C from *Alcaligenes sp.*	3	61
16	Lipase B from *Alcaligenes sp.*	3	71
17	CAL-B (immob)	3	94
18	*Candida antarctica* Lipase B (liq.)	0	-[d]

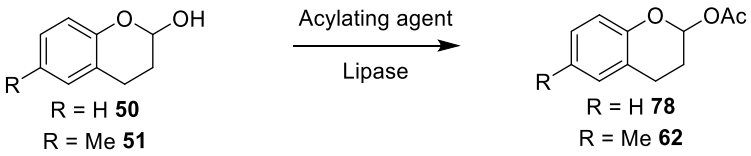
The following lipases gave conversion <2% and were not subjected to HPLC analysis: Hog pancreas lipase, Acid protease A, Protease from *Aspergillus niger*, Lipase from *Aspergillus niger*, Lipase A from *Alcaligenes sp*, Neutral Protease A, Lipase from *Rhizomucor miehei.* The following enzymes gave no conversion: Lipase from fungal source, Protease A from *Bacillus subtilis*, Phytase, Alkaline protease A, Alkaline Lipase A, Lipase from *Bromeliaceae sp,* Lipase from *Carica papaya*, Protease A from *Aspergillus oryzae*, Protease B from *Bacillus subtilis*, Acylase from *Aspergillus sp*, Lipase from *Rhizopus niveus*, Protease from *Bacillus stearothermophilus*, Lipase from *Penicillum roquefort*, Protease from *Aspergillus melleus*, Lipase from *Penicillum camembertii*, Protease B from *Aspergillus oryzae*, Protease A from *Bacillus sp*, Protease B from *Bacillus sp*, Lipase A from *Rhizomucor miehei*, Lipase Porcine Pancrease type II, Lipase from *Penicillum roquefort*, Protease C from *Bacillus subtilis*, Alkaline protease B, Proteax, Lipase from *Pseudomonas fluorescens*, Lipase A from *Burkholderia cepacia.* [a]Conversion was determined by ^{1}H NMR comparing the signals for the C(2) proton in each compound; [b]enantiomeric excess was determined by chiral HPLC and is the excess of the second enantiomer detected in all cases which has retention time of approx. 6.4 mins; [c]two different samples of *Candida rugosa* lipase B were tested; [d]compounds with conversion <2% were not subjected to chiral HPLC analysis.

[1]*Candida rugosa* has been renamed to *Candida cylindracea* but will be referred to as *Candida rugosa* in this work, as two different samples of lipases were used.

Candida antarctica lipase B (CAL-B) (immobilised) furnished excellent selectivity (94% *ee*, Entry 17) albeit with very low conversion. The non-immobilised *Candida antarctica* lipase B (Entry 18) gave no conversion, but the mixture of lipases from this organism (Entry 13) performed similarly to the immobilised CAL-B, in terms of both selectivity (93% *ee*) and conversion. Interestingly, Lipase A from *Candida antarctica* (Entry 4), which is present in the mixture of lipases from this organism, gave good conversion but with poor selectivity. A follow-up investigation was carried out, where the acylating agent was varied, in order to see if this would affect the selectivity and conversion.

Interestingly, for the enzymes shown in Table 3.3, the enantioselectivities are very similar across the two compounds **50** and **51**, but the conversions are lower for the substituted compound **51**, showing that the methyl group may not be easily accommodated in the active site.

*Table 3.3 Comparison of acylation data for the unsubstituted lactol **50**, and the 6 methyl lactol **51***



R = H **50**
R = Me **51**

R = H **78**
R = Me **62**

Entry	Hydrolase	50[a]		51	
		Conv. (%)[b]	*ee* (%)[c]	Conv. (%)[b]	*ee* (%)[c]
1	CAL-B (immob)	20	95	3	97
2	Lipase E from *Alcaligenes sp.*	47	77	30	70
3	Lipase A from *Candida rugosa*	25	60	26	64
4	*Lipase from Thermomyces lanuginosus*	-[d]	-[d]	12	97
5	Lipase B from *Burkholderia cepacia*	44	78	5	74
6	Lipase from *Pseudomonas cepacia*	19	65	3	80

[a]Reported by Gavin et al.;[4] [b]conversion was determined by ^{1}H NMR; [c]enantiomeric excess was determined by chiral HPLC analysis; [d]this enzyme was not tested for activity against **50**.

3.4.4 *Candida Antarctica* lipase experiments

Disappointingly CAL-B (immob) gave poor conversion, but showed excellent enantioselectivity (Table 3.1, Entry 17). As a result of the poor conversion, when using the *Candida antarctica* lipases, it was decided to repeat the 24 h screens using different acylating agents, shown in Figure 3.6. The acylating agents used were vinyl acetate **65**, ethyl acetate **79**, isopropyl acetate **80**, and isopropenyl acetate **68**.

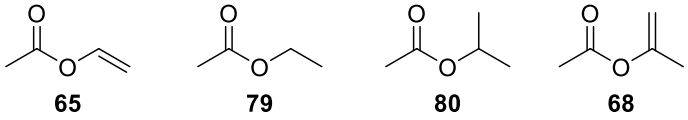


Figure 3.6 The structures of acylating agents used in this study

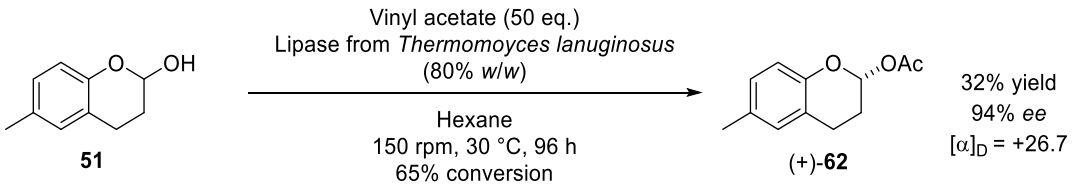
Changing the acylating agent showed a slight improvement in conversion values in some cases but no reaction in other cases (Table 3.4). Overall the different acylating agents showed little to no enhancement of the reaction over the use of vinyl acetate **33**. Acetaldehyde **67** is known to inactivate some lipases through formation of a Schiff base with the lysine residues of the protein.[51]

3.4.5 Solvent screen

Following the initial screens, enzymes which showed good conversion and enantioselectivity were subjected to further screens, using a range of organic solvents. Organic solvents are known to increase lipase selectivity by interfacial activation.

Six solvents were screened: hexane (HPLC grade), heptane (HPLC grade), diisopropyl ether, *tert*-butyl methyl ether (HPLC grade), and toluene (dry, distilled). Four lead enzymes were selected for screening on the basis of activity. Enzymes which had poor selectivity were disregarded. The solvent screens were carried out at 30°C and 150 rpm. The results and conditions are shown in Table 3.5.

The lipase from *Thermomyces lanuginosus* showed excellent selectivity (>92%) in the solvent screen, especially with the use of hexane and toluene (Entry 3). A preparative scale synthesis was carried out, using hexane as solvent (Scheme 3.16). After 96 h, the ^{1}H NMR spectrum showed 65% conversion to the product, and, after chromatography, the acetate **62** was isolated in 32% yield with 94% *ee*.



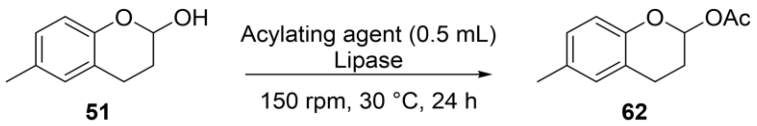
*Scheme 3.16 Preparative scale resolution of **51***

In the case of the unsubstituted compound **50**, the use of toluene and hexane gave the best results in the solvent screen, which was also observed here. With the exception of

Thermomyces lanuginosus the presence of the polar solvents, TBME and DIPE, decreased the conversion relative to the control reaction (vinyl acetate only), albeit, giving the highest enantioselectivities for these lipases. Previous reports of transesterification using vinyl acetate have shown that non-polar solvents, especially hexane, give superior results compared to polar solvents.[52]

Thermomyces lanuginosus has previously used in the production of biodiesel from soybean oil,[53] evolved for the use in the synthesis of pregabalin,[54] and has been reviewed in the context of use in an industrial setting.[55]

*Table 3.4 Investigation of the effect of the acylating agent on the resolution of **51***

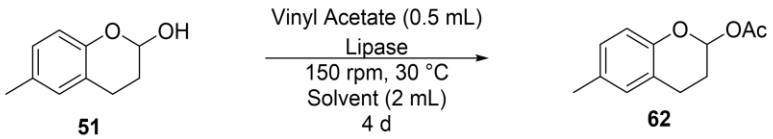


51 **62**

Entry	Enzyme	Vinyl acetate		Ethyl acetate[a]		Isopropyl acetate		Isopropenyl acetate	
		Conv. (%)[b]	*ee* (%)[c]	Conv. (%)[b]	*ee* (%)[c]	Conv. (%)[b]	*ee* (%)[c]	Conv. (%)[b]	*ee* (%)[c]
1	CAL-B (immob)	3	94	1	-[d]	4	94	1	96
2	Lipase from *Candida antarctica*	4	93	1	-[d]	3	93	3	95
3	*Candida antarctica* Lipase A	26	8	1	-[d]	-	-[d]	2	15
4	*Candida antarctica* Lipase B (liq.)	0	-[d]	0	-[d]	0	-[d]	0	-[d]

In the absence of any lipase there was no conversion to the product. [a]The ethyl acetate reaction time was 72 h; [b]conversion was determined by ^{1}H NMR comparing the signals for the C(2) proton in each compound. [c]Enantiomeric excess was determined by chiral HPLC and the second enantiomer eluted is the major enantiomer detected in all cases which has retention time of approx. 6.4 mins; [d]compounds with conversion <2% were not subjected to chiral HPLC analysis

*Table 3.5 Solvent screen results for 6-methyl lactol **51***



51 **62**

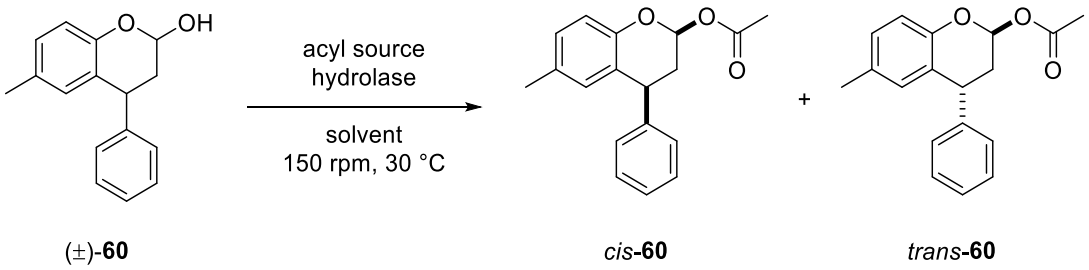
Entry	Enzyme	no solvent[a]		Hexane		Heptane		Toluene		DIPE		TBME	
		Conv (%)[b]	*ee* (%)[c]	Conv (%)[b]	*ee* (%)[c]	Conv (%)[b]	*ee* (%)[c]	Conv (%)[b]	*ee* (%)[c]	Conv (%)[b]	*ee* (%)[c]	Conv (%)[b]	*ee* (%)[c]
1	Lipase E from *Alcaligenes sp*	57	67	>95	44	58	58	53	62	6	67	21	67
2	Lipase A from *Candida rugosa*	38	60	48	67	18	59	26	69	13	64	13	79
3	Lipase from *Thermomyces lanuginosus*	13	94	86	92	38	95	55	94	27	96	51	94
4	Lipase from *Candida cylindracea*	29	66	63	66	41	63	30	73	18	68	5	83

Reactions carried out in the absence of an enzyme gave no conversion to product. [a]Reaction carried out using 0.5 mL vinyl acetate as acylating agent and solvent; [b]conversion was determined by ^{1}H NMR; [c]enantiomeric excess was determined by chiral HPLC anaylsis.

3.5 Conclusion

We have demonstrated lipase-mediated dynamic kinetic resolution of a fused lactol system.

Extension to this work was carried out, wherein the tolterodine lactol **60**, containing a remote stereocentre, was treated with hydrolases, which proved more challenging in terms of efficiency, while retaining high enantioselectivity (up to >98% *ee*).[4]



(±)-**60** *cis*-**60** *trans*-**60**

*Scheme 3.17 Resolution of the Tolterodine intermediate lactol **60***

3.6 References

1. Arora, R. B.; Mathur, C. N. *Br. J. Pharmacol.* 1963; *20*:29-35.
2. Lacy, A. *Curr. Pharm. Des.* 2004; *10*:3797-811.
3. Kumari, G. N.; Ganesh, M. R.; Anitha, R.; Sivasubramanian, A. *Z. Naturforsch. C.* 2004; *59*:405-7.
4. Gavin, D. P.; Foley, A.; Moody, T. S.; Rao Khandavilli, U. B.; Lawrence, S. E.; O'Neill, P.; Maguire, A. R. *Tetrahedron: Asymmetry* 2017; *28*:577-85.
5. Villo, L.; Kreen, M.; Kudryashova, M.; Metsala, A.; Tamp, S.; Lille, Ü.; Pehk, T.; Parve, O. *J. Mol. Catal. B: Enzym.* 2011; *68*:44-51.
6. Villo, L.; Danilas, K.; Metsala, A.; Kreen, M.; Vallikivi, I.; Vija, S.; Pehk, T.; Saso, L.; Parve, O. *J. Org. Chem.* 2007; *72*:5813-6.
7. Villo, L.; Metsala, A.; Parve, O.; Pehk, T. *Tetrahedron Lett.* 2002; *43*:3203–7.
8. van den Heuvel, M.; Cuiper, A. D.; van der Deen, H.; Kellogg, R. M.; Feringa, B. L. *Tetrahedron Lett.* 1997; *38*:1655-8.
9. van der Deen, H.; Cuiper, A. D.; Hof, R. P.; van Oeveren, A.; Feringa, B. L.; Kellogg, R. M. *J. Am. Chem. Soc.* 1996; *118*:3801-3.
10. Prabhakar, S.; Vives, T.; Ferrieres, V.; Benvegnu, T.; Legentil, L.; Lemiegre, L. *Green Chem.* 2017; *19*:987-95.
11. Villo, L.; Metsala, A.; Tamp, S.; Parve, J.; Vallikivi, I.; Järving, I.; Samel, N.; Lille, Ü.; Pehk, T.; Parve, O. *ChemCatChem* 2014; *6*:1998-2010.
12. Sabbani, S.; Hedenstrom, E.; Andersson, J. *Tetrahedron: Asymmetry* 2007; *18*:1712-20.
13. Chênevert, R.; Pelchat, N.; Morin, P. *Tetrahedron: Asymmetry* 2009; *20*:1191-6.
14. Szymanski, W.; Ostaszewski, R. *J. Mol. Catal. B: Enzym.* 2007; *47*:125-8.
15. Kamal, A.; Sandbhor, M.; Ahmed, K.; Adil, S. F.; Shaik, A. A. *Tetrahedron: Asymmetry* 2003; *14*:3861-6.
16. Akai, S.; Naka, T.; Fujita, T.; Takebe, Y.; Kita, Y. *Chem. Commun.* 2000; *16*:1461-2.
17. Brodzka, A.; Koszelewski, D.; Ostaszewski, R. *J. Mol. Catal. B: Enzym.* 2012; *82*:96-101.
18. Schnell, B.; Strauss, U. T.; Verdino, P.; Faber, K.; Kappe, C. O. *Tetrahedron: Asymmetry* 2000; *11*:1449-53.
19. Klomklao, T.; Pyne, S. G.; Baramee, A.; Skelton, B. W.; White, A. H. *Tetrahedron: Asymmetry* 2003; *14*:3885-9.
20. Bailey, G. C.; Boettner, F. *J. Ind. Eng. Chem.* 1921; *13*:905-6.
21. Pickett, J. E.; Vandort, P. C. *Tetrahedron Lett.* 1992; *33*:1161-4.
22. Sato, K.; Amakasu, T.; Abe, S. *J. Org. Chem.* 1964; *29*:2971-2.
23. Panetta, J. A.; Rapoport, H. *J. Org. Chem.* 1982; *47*:946-50.
24. Kim, D.; Min, M.; Hong, S. *Chem. Commun.* 2013; *49*:4021-3.
25. Kamat, D. P.; Tilve, S. G.; Kamat, V. P. *Tetrahedron Lett.* 2012; *53*:4469-72.
26. Zeitler, K.; Rose, C. A. *J. Org. Chem.* 2009; *74*:1759-62.
27. Gu, Y.; Xue, K. *Tetrahedron Lett.* 2010; *51*:192-6.
28. Mandal, S.; Mahato, S.; Jana, C. K. *Org. Lett.* 2015; *17*:3762-5.
29. Haser, K.; Wenk, H. H.; Schwab, W. *J. Agric. Food Chem.* 2006; *54*:6236-40.
30. Dirat, O.; Bibb, A. J.; Burns, C. M.; Checksfield, G. D.; Dillon, B. R.; Field, S. E.; Fussell, S. J.; Green, S. P.; Mason, C.; Mathew, J.; Mathew, S.; Moses, I. B.; Nikiforov, P. I.; Pettman, A. J.; Susanne, F. *Org. Process Res. Dev.* 2011; *15*:1010-7.
31. De Castro, K. A.; Ko, J.; Park, D.; Park, S.; Rhee, H. *Org. Process Res. Dev.* 2007; *11*:918-21.

32. Srinivas, K.; Srinivasan, N.; Reddy, K. S.; Ramakrishna, M.; Reddy, C. R.; Arunagiri, M.; Kumari, R. L.; Venkataraman, S.; Mathad, V. T. *Org. Process Res. Dev.* 2005; *9*:314-8.
33. Andersson, P. G.; Schink, H. E.; Österlund, K. *J. Org. Chem.* 1998; *63*:8067-70.
34. Selenski, C.; Pettus, T. R. *J. Org. Chem.* 2004; *69*:9196-203.
35. Chen, G.; Tokunaga, N.; Hayashi, T. *Org. Lett.* 2005; *7*:2285-8.
36. Gallagher, B. D.; Taft, B. R.; Lipshutz, B. H. *Org. Lett.* 2009; *11*:5374-7.
37. Luo, Y.; Carnell, A. J. *Angew. Chem. Int. Ed.* 2010; *49*:2750-4.
38. Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. *J. Am. Chem. Soc.* 1988; *110*:7200-5.
39. Pelagalli, R.; Chiarotto, I.; Feroci, M.; Vecchio, S. *Green Chem.* 2012; *14*:2251.
40. Wang, Y.; Wang, R.; Li, Q.; Zhang, Z.; Feng, Y. *J. Mol. Catal. B: Enzym.* 2009; *56*:142-5.
41. Bouzemi, N.; Debbeche, H.; Aribi-Zouioueche, L.; Fiaud, J.-C. *Tetrahedron Lett.* 2004; *45*:627-30.
42. Xu, G.; Chen, Y.; Wu, J.; Cheng, Y.; Yang, L. *Tetrahedron: Asymmetry* 2011; *22*:1373-8.
43. Irimescu, R.; Saito, T.; Kato, K. *J. Mol. Catal. B: Enzym.* 2004; *27*:69-73.
44. Mezzetti, A.; Keith, C.; Kazlauskas, R. J. *Tetrahedron: Asymmetry* 2003; *14*:3917-24.
45. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* 2000; *11*:1199-210.
46. Biswas, B.; Sen, P. K.; Venkateswaran, R. V. *Tetrahedron* 2007; *63*:12026-36.
47. Peters, M.; Trobe, M.; Tan, H.; Kleineweischede, R.; Breinbauer, R. *Chem. Eur. J.* 2013; *19*:2442-9.
48. Liang, H.; Ciufolini, M. A. *Chem. Eur. J.* 2010; *16*:13262-70.
49. Yates, P.; Macas, T. S. *Can. J. Chem.* 1988; *66*:1-10.
50. Deasy, R. E. Ph.D. Thesis, NUI Cork, 2012.
51. Franken, B.; Eggert, T.; Jaeger, K. E.; Pohl, M. *BMC Biochem.* 2011; *12*:10.
52. Yadav, G. D.; Trivedi, A. H. *Enzyme Microb. Technol.* 2003; *32*:783-9.
53. Mukherjee, J.; Gupta, M. N. *Biotechnol. Rep. (Amst)* 2016; *10*:38-43.
54. Zheng, R. C.; Ruan, L. T.; Ma, H. Y.; Tang, X. L.; Zheng, Y. G. *Biochem. Eng. J.* 2016; *113*:12-8.
55. Fernandez-Lafuente, R. *J. Mol. Catal. B: Enzym.* 2010; *62*:197-212.

Chapter 4

Towards dynamic kinetic resolution in the intramolecular nitroaldol (Henry) reaction through lipase catalysis

Contents

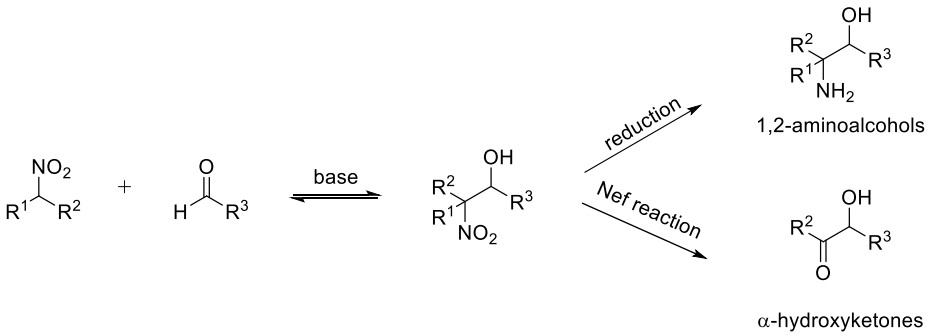
Overview

The work described in this chapter builds on the work carried out by Deasy during her PhD, and subsequent work carried out by Milner and Gavin.[1,2] The introduction will consist of an overview of the work completed by Deasy, Milner and Gavin predating the work in this chapter, which, when combined, led to a publication.[2] This chapter will outline the work completed and will be similar to the publication; synthesis of substrates will be discussed in more detail, and the material which had been moved to the supplementary material for publication will be discussed in more detail.

Dynamic kinetic resolution (DKR) in the intramolecular nitroaldol reaction through coupling with lipase-mediated acetylation is described herein. Significant challenges in effecting the combination of the base-mediated reversible cyclisation of 6-nitroheptanal **81** with the selective enzyme-mediated transesterification were encountered. Ultimately, *trans*-2-methyl-2-nitrocyclohexyl acetate **82b** was isolated in excellent enantiopurity (>98% *ee*) *via* a sequential DKR sequence where the enzymatic resolution and base-mediated interconversion of the 2-methyl-2-nitrocyclohexanol **83** were effected alternately, demonstrating the feasibility of this approach for the first time. Further work showed, for the first time, evidence that a DKR-type system is possible: reaction engineering allowed design of a sequential one-pot reaction system which furnished the products with excellent enantioselectivity, and good diastereoselectivity.

4.1 Introduction

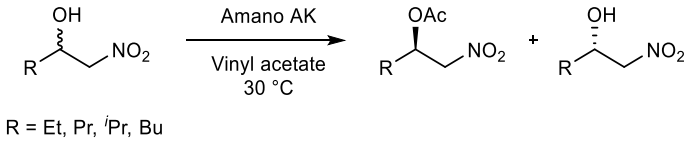
The Henry reaction is an important base-mediated transformation in organic chemistry leading to vicinal nitro alcohols which can be converted to a wide variety of synthetic intermediates, such as 1,2-aminoalcohols and α-hydroxycarbonyl compounds (Scheme 4.1).[3-5] Although the reaction is known for over a century, stereo- and diastereoselective approaches leading to enantiopure nitroalcohols are still challenging. Principal approaches to the catalytic asymmetric nitroaldol reaction, including transition metal and organo-catalysed methods, have been reviewed in detail.[5-7] The use of biocatalytic protocols to resolve the products of the Henry reaction has also been reviewed.[8]



Scheme 4.1 Henry reaction and synthesis of 1,2-aminoalcohols & α-hydroxyalcohols

There are two distinct biocatalytic methods: direct enzyme-catalysed asymmetric Henry reaction using hydroxynitrile lyases, or initial formation of the β-nitroalcohol product, followed by enzymatic kinetic resolution of the resulting stereoisomers.[9] The latter kinetic resolution suffers the limitation of a maximum attainable yield of 50% of the desired product.

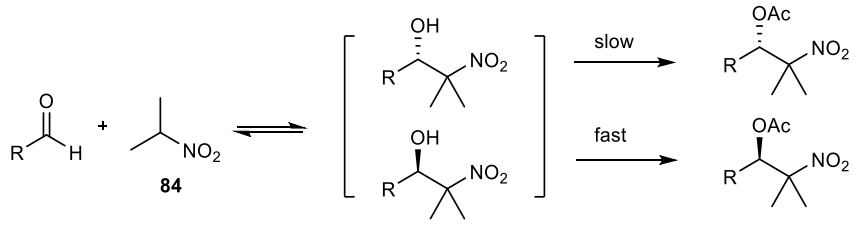
Okamoto et al. reported the first bioresolution of the products of a Henry reaction (Scheme 4.2). The use of various solvents was explored, and isopropyl ether was found to give good results.[10]



Scheme 4.2 Resolution of Henry reaction products.

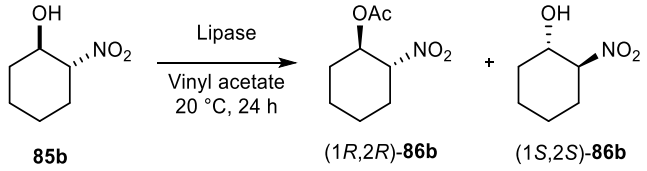
Ramström et al. developed a procedure for the one-pot intermolecular dynamic kinetic resolution of β-nitroalcohols; this was limited by the need to use a large excess of the

nitroalkane **84** in order to shift the equilibrium towards the formation of the product (Scheme 4.3).[11,12]



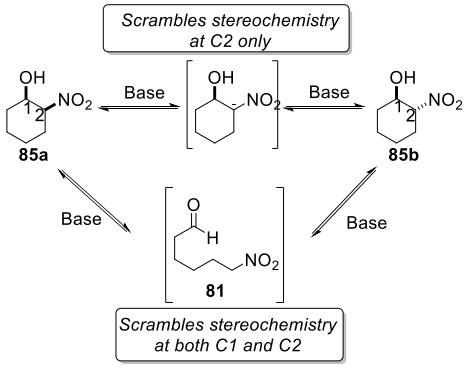
Scheme 4.3 One-pot intermolecular Henry reaction and subsequent lipase catalysed resolution

Previous work in the Maguire group had the added challenge of a second stereocentre, leading to potential complications in stereocontrol and diastereoselectivity (Scheme 4.4). We have previously reported efficient kinetic bioresolution for both the *cis*- and *trans*-2-nitrocyclohexanols **85a** and **85b** via enzyme-mediated transesterification.[13]



*Scheme 4.4 Resolution of 2-nitrocyclohexanol **85b***

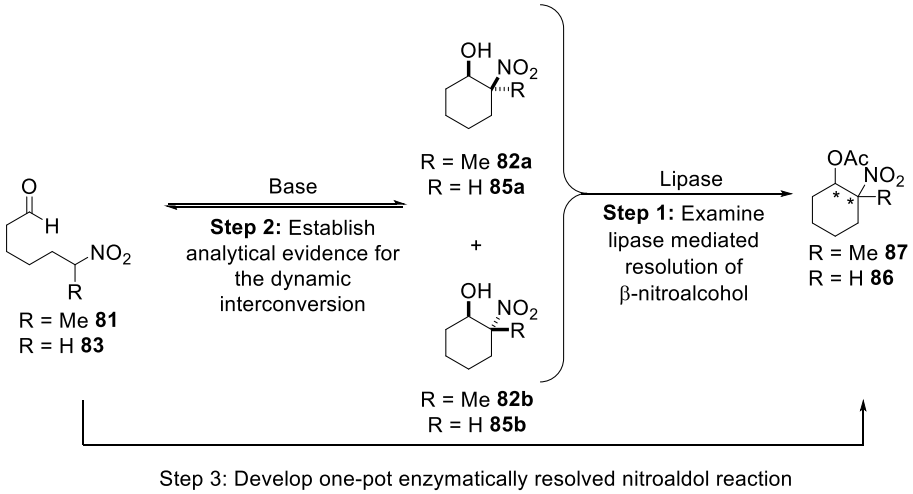
Initially, Milner reported the lipase-mediated resolution of nitrocyclohexanol **85a** and **85b**.[13] When the base-mediated interconversion was attempted using this substrate, stereochemical scrambling was only observed at one stereocentre. This was rationalised as base-catalysed epimerisation (Scheme 4.5). To overcome this problem, a methyl group was installed at the acidic site to block the epimerisation pathway. As a result, the base-catalysed interconversion through ring opening and closing was the only available reaction pathway, allowing the possibility of combining the two processes.



Scheme 4.5 Epimerisation pathway versus the reversible nitroaldol reactions

Crucial to the success of the lipase-mediated DKR protocol is selection of the correct base. The fundamental requirements of the selected base are firstly to mediate effective ring closure of 6-nitroheptanal **81** and then catalyse the dynamic interconversion process between the diastereomeric alcohols **82a** and **82b.** It was then envisaged that combination with the diastereoselective lipase-mediated transesterification (as discussed above) would lead to a one-pot DKR of the intramolecular nitroaldol reaction through lipase catalysis.

Deasy initially screened the substrate **83** against a variety of bases to identify the best base for the interconversion using vinyl acetate as the solvent; ultimately, combination of the two systems would require the use of the base and the acylating agent concurrently (Scheme 4.6).[1]



Scheme 4.6 Investigation of the intramolecular nitroaldol reaction in a stepwise manner.

Deasy showed that the use of piperidine (2 eq.) and aqueous sodium hydroxide (1M) gave no ring closure of the aldehyde **83**. Diethylamine (DEA) and Hünig's base gave <20% conversion to the alcohols **82a** and **82b**. Triethylamine and DABCO both catalysed the ring closing reaction but did not catalyse the reversible interconversion of **82a** and **82b**.

The use of DBU (0.1 eq.) gave complete cyclisation of the aldehyde in 24 hours, albeit producing a large amount of the acetates **87a** and **87b**, racemically. When the loading was reduced to 0.05 eq. and the reaction time extended from 24 hours to 48 hours, 50% of aldehyde 1 remained, but only 3% of the mixture was acetates **87a** and **87b**.

Tetramethylguanidine (TMG) was also used in the cyclisation, using 0.1 eq., resulting in a sluggish reaction, giving only 31% conversion to the alcohols **82a** and **82b**, after 24 hours; an

additional 2 equivalents of TMG gave better conversion, with only 6% of the aldehyde remaining after 48 hours, albeit with 5% of acetate **87a**.

Deasy also showed experimentally, that interconversion of the alcohol **82a** to **82b** and vice versa was taking place in the presence of 0.1 eq. DBU; this was carried out in $CDCl_3$ so that the interconversion could be monitored by ^1H NMR. After 24 hours, the alcohols **82a** and **82b** were in the ratio 33:67, regardless of which diastereomer was added initially. The interconversion was also carried out using TMG (2.0 eq.), however, it took 72 hours to get to the same ratio.

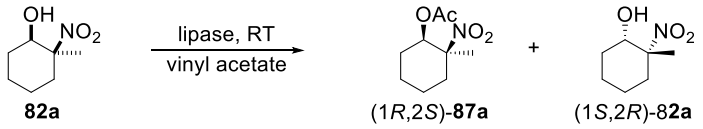
The bioresolution of an intramolecular nitroaldol reaction of 6-nitroheptanal **81** was investigated in a stepwise manner (Scheme 4.6). The first step in this study involved independent examination of the lipase-mediated kinetic resolution of the racemic *cis*- and *trans*-2-methyl-2-nitrocyclohexanols **82a** and **82b** establishing the most efficient lipase to perform this biotransformation diastereoselectively. Ideally, for an efficient dynamic process, one enantiomer of either **82a** or **82b** would be efficiently and selectively acetylated. The optimum reaction conditions reported for the kinetic resolution of *cis*- and *trans*-2-nitrocyclohexanol **85a** and **85b** employing vinyl acetate as both solvent and acyl donor were applied in this study for the lipase-mediated transesterification of **82**.[13]

Deasy examined the resolution of each alcohol diastereomer separately using a variety of lipases. A selection of the results is shown in Table 4.1 (*cis*-**82a**)[2] and Table 4.2 (*trans*-**82b**).

P. fluorescens furnished the product acetate *cis*-**87a** with good conversion (40%) with high enantiopurity (96% *ee*), compared to the *trans*-**87b** product, which had conversion <10%, under the same conditions (Table 4.1, Entry 3 and Table 4.2, Entry 3, respectively). The diastereoselectivity here was good, but the extended reaction time (113.5 h) was too long to be useful. *P. stutzeri*, after 48 hours, furnished *trans*-**87b** with 50% conversion, and excellent enantioselectivity (<98% *ee*), however, after the same period of time, its use gave the acetate *cis*-**87b** with 78% conversion, but with poor enantiopurity (27% *ee*), which was accompanied by excellent enantiopurity of the alcohol *cis*-**87a** (>98%) (Table 4.1, entry 2 and Table 4.2, entry 2).

[2]Throughout this work "*cis*" and "*trans*" are used referring to "seq-cis" and "seq-trans" i.e. the assignment of stereochemistry is based on the sequence rule (Cahn-Ingold-Prelog rules).

*Table 4.1 Lipase-mediated transesterification of cis-2-methyl-2-nitrocyclohexanol **82a** in vinyl acetate*

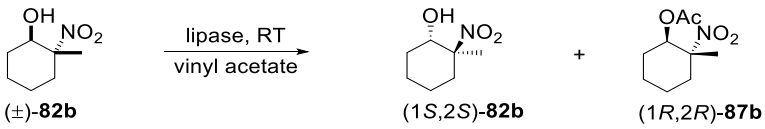


82a (1*R*,2*S*)-**87a** (1*S*,2*R*)-**82a**

Entry	Enzyme Source	Time (h)	Conversion (%)	*ee* (%)[a] Alcohol *cis*-**82a** (1*S*,2*R*)	*ee* (%)[a] Acetate *cis*-**87a** (1*R*,2*S*)	E[b]
1	CAL-B (immob)	72	33[b]	49	>98	159
2	*Pseudomonas stutzeri*	48	78[b]	>98	27	6.4
3	*Pseudomonas fluorescens*	113.5	40[b]	64	96	95

Adapted from Deasy.[1] [a]Enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [b]conversion and the enantiomeric ratio (E value) were calculated from the enantiomeric excess of substrate alcohol **82a** (ee_s) and product acetate **87a** (ee_p);[15]

Table 4.2 Lipase-mediated transesterification of trans*-2-methyl-2-nitrocyclohexanol **82b** in vinyl acetate*



(±)-**82b** (1*S*,2*S*)-**82b** (1*R*,2*R*)-**87b**

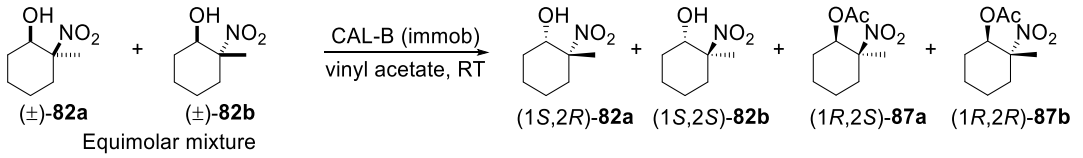
Entry	Enzyme Source	Reaction Time (h)	Conversion (%)	*ee* (%)[a] Alcohol *trans*-**82b** (1*S*,2*S*)	*ee* (%)[a] Acetate *trans*-**87b** (1*R*,2*R*)	E[b]
1	CAL-B (immob)	72	49[b]	96	>98	>200
2	*Pseudomonas stutzeri*	48	50[b]	97	>98	>200
3	*Pseudomonas fluorescens*	113.5	<10[c]	–	–	–

Adapted from Deasy.[1] [a]Enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [b]conversion and the enantiomeric ratio (E value) were calculated from the enantiomeric excess of substrate alcohol **82b** (ee_s) and product acetate **87b** (ee_p);[14] [c]conversion was determined by ^{1}H NMR spectroscopy of the crude products to be <10%, chiral HPLC analysis was not conducted.

Candida antarctica lipase B (CAL-B) (immob) showed only moderate diastereoselectivity, furnishing *trans*-**87b** with 49% conversion, and *cis*-**87a** with 33% conversion, both with >98% *ee*, after 72 hours (Table 4.1, entry 1 and Table 4.2, entry 1). Further reaction using an equimolar mixture of both alcohols *cis*-**82a** and *trans*-**82b** showed that the diastereoselectivity was much better with short reaction times than after longer reaction times, albeit with lower conversion than longer reaction times (Table 4.3). This shows that one diastereomer, *trans*-**82b**, can be acylated preferentially, with excellent enantioselectivity. In the case where this is combined with an effective base-mediated interconversion, the interconversion between the

two diastereomers, constantly regenerating the trans isomer, *trans*-**82b**, should effectively overcome decreased diastereoselectivity after extended reaction time.

Table 4.3 Diastereoselective CAL-B (immob) mediated transesterification of cis-***2a*** *and* trans-***2b*** *in vinyl acetate*



(±)-**82a** (±)-**82b** Equimolar mixture

(1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

Enzyme Source	Reaction Time (h)	Alcohol 82		Acetate 87	
		cis-**82a** (%)[a]	*trans*-**82b** (%)[a]	*cis*-**87a** (%)[a]	*trans*-**87b** (%)[a]
CAL-B (immob)	12	44	38	3	15
	18.5	43	36	4	17
	40.5	41	30	6	23
	62.5	27	30	11	32
		(15% *ee*)[b,c]	(74% *ee*)[b,d]	(>98% *ee*)[b,e]	(>98% *ee*)[b,f]

Adapted from Deasy[1] [a]Conversions were estimated by ^{1}H NMR spectroscopy and were derived from integration of the ^{1}H NMR spectrum of the mixture of the crude material, not mass recovery. Starting material was an equimolar mixture of *cis*-**82a** and *trans*-**82b**; [b]enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [c]the principal enantiomer was (1*S*,2*R*)-**82a**; [d]the principal enantiomer was (1*S*,2*S*)-**82b**; [e]the principal enantiomer was (1*R*,2*S*)-**87a**; [f]the principal enantiomer was (1*R*,2*R*)-**87b**.

Having established a diastereoselective enzymatic resolution, attention next focused on the aldehyde cyclisation and the interconversion. Deasy tested a variety of bases, summarised in Table 4.4. Piperidine, aqueous sodium hydroxide, diethylamine (DEA), and *N*,*N*-diisopropylethylamine (Hünig's base) showed little efficiency in the cyclisation of **81**, even at 2.0 eq. loading (Table 4.4, Entries 1–5), and their use in the base-mediated dynamic interconversion process was not further pursued.

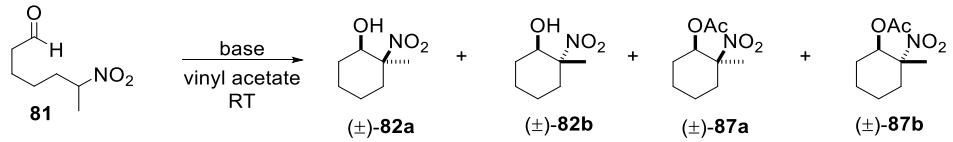
Both triethylamine and DABCO (Entries 5 and 6) successfully catalysed the nitroaldol reaction, without any competing chemical acylation. When used in the base-catalysed interconversion of *cis*-**82a** and *trans*-**82b** there was no evidence of interconversion.

The TMG-mediated dynamic interconversion process was also explored. On exposure of the more stable *trans*-**82b** diastereomer to TMG (2.0 eq.) the *cis*-**82a** diastereomer was observed within 1 h of the initial addition of the TMG and a thermodynamic ratio (*cis*-**82a**:*trans*-**82b** 26:74) was attained after 5 h (Table 4.5).

Using DBU as base successfully catalysed the cyclisation of **81** at low loadings (Table 4.5, Entries 7–9), albeit with a large extent of chemical acylation evident. Although there was a large extent of chemical acylation evident, it was hoped that on combination of the lipase-

catalysed resolution and the base-catalysed interconversion, that the resolution would effectively compete with the chemical acylation.

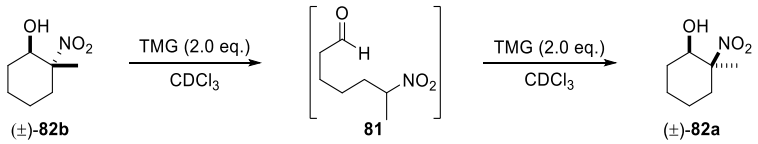
*Table 4.4 Base mediated cyclisation of aldehyde **81***



Entry	Base	Eq.	Time (h)	Aldehyde 81 (%)[a]	Alcohol 82		Acetate 87	
					cis-82a (%)[a]	*trans*-82b (%)[a]	*cis*-87a (%)[a]	*trans*-87b (%)[a]
1	Piperidine	2.0	48	100	–	–	–	–
2	NaOH (1M)	–	48	100	–	–	–	–
3	DEA	2.0	48	82	8	10	–	–
4	Hünig's Base	2.0	48	85	5	10	–	–
5	NEt_3[b]	2.0	72	9	24	67	–	–
6	DABCO	2.0	48	–	40	60	–	–
7	DBU	0.1	24	–	11	52	22	15
8	DBU	0.1	48	–	–	30	37	33
9	DBU	0.05	48	50	16	31	2	1
10	TMG	0.1	24	69	10	21	–	–
11	TMG	0.1–2.1[c]	48	6	30	57	5	–

Adapted from Deasy.[1] [a]Conversions were determined by ^{1}H NMR spectroscopy and were derived from integration of the ^{1}H NMR spectrum of the mixture of the crude material not mass recovery; [b]carried out at 40°C; [c]At 24 h after analysis by ^{1}H NMR spectroscopy an additional 2.0 eq. of TMG were added to the reaction vessel and stirred at room temperature for a further 24 h.

Table 4.5 Evidence for dynamic interconversion – trans*-2-methyl-2-nitrocyclohexanol **82b** and TMG (2.0 eq.) in $CDCl_3$*



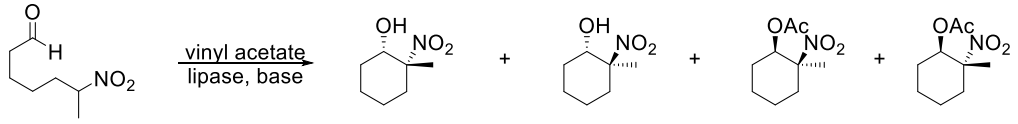
Reaction Time (h)	82a (%)[a]	82b (%)[a]
0	-	100
0.8	14	88
5.1	26	74
72	28	72

Adapted from Deasy.[1] [a]6-Nitroheptanal **81** was not detected in the ^{1}H NMR spectra.

When DBU (0.1 eq.) was combined with CAL-B (immob), the intramolecular cyclisation did not proceed, returning only starting material after 72 h (Table 4.6, Entry 1). The use of *P. stutzeri* with this base effectively mediated the ring-closing, however, poor enantioselectivity in the resolution meant this was not a viable one-pot system (Entries 2 and 3). When the base was

changed to TMG, the ring-closing proceeded sluggishly, with aldehyde **81** (15%) still evident after 48 hours; and the low extent of conversion to the products *cis*-**87a** and *trans*-**87b**, was disappointing (Entry 4).

Table 4.6 Combination of the lipase-catalysed resolution and the base-catalysed ring closure/interconversion



81 (1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

Entry	Base (eq.)	Lipase Source	Reaction Time (h)	Aldehyde 81 (%)[a]	Alcohol 82		Acetate 87	
					cis-**82a** (%)[a] [*ee* (%)][b,c]	*trans*-**82b** (%)[a] [*ee* (%)][b,d]	*cis*-**87a** (%)[a] [*ee* (%)][b,e]	*trans*-**87b** (%)[a] [*ee* (%)][b,f]
1	DBU (0.1)	CAL-B (immob)	72	100	–	–	–	–
2	DBU (0.5)	*P. stutzeri*	48	–	6 [>98][g]	4 [>98][g]	37 [1]	53 [1]
3	DBU (1.0)	*P. stutzeri*	48	–	6 [>98][g]	3 [>98][g]	53 [1]	38 [0]
4	TMG (2.0)	*P. stutzeri*	48	15	22 [35]	50 [6]	9 [80]	4 [75]

Adapted from Foley et al. (supporting information).[2] [a]Conversions were determined by ^{1}H NMR spectroscopy and were derived from integration of the ^{1}H NMR spectrum of the mixture of the crude material, not mass recovery; [b]enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [c]the principal enantiomer was (1*S*,2*R*)-**82a**; [d]the principal enantiomer was (1*S*,2*S*)-**82b**; [e]the principal enantiomer was (1*R*,2*S*)-**87a**; [f]the principal enantiomer was (1*R*,2*R*)-**87b**; [g]while chiral HPLC indicates high enantiomeric excess for the recovered alcohols (1*S*,2*R*)-**82a** and (1*S*,2*S*)-**82b**, the very low levels present mean the enantiopurity should be interpreted with caution.

Both the reversible ring opening/closing and the resolution worked separately, but when the two reaction were combined, neither reaction worked effectively. The use of the base and the lipase concurrently was not viable; therefore, it was decided to carry out the interconversion and the resolution consecutively, in a two-pot reaction. This overcame the base-lipase inhibition by separating the reagents and allowed the use of neat vinyl acetate as the solvent for the resolution (Figure 4.7). The problems of chemical acylation, and base-lipase inhibition were overcome by physically separating the two reactions. This ultimately returned the preferred product *trans*-**87b** with excellent enantiopurity (>98% *ee*) which made up 57% of the reaction mixture, after three cycles.

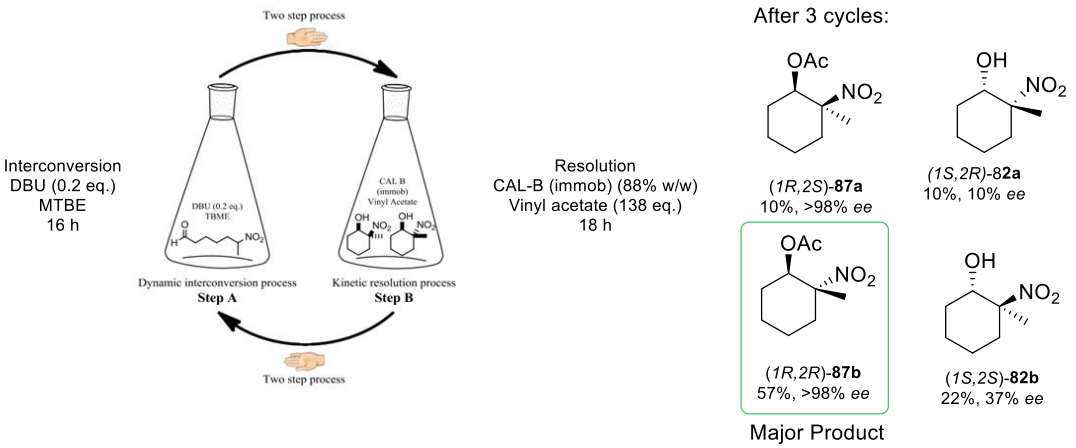


Figure 4.7 Two-pot system developed by Deasy, % composition determined by ^{1}H NMR.[1,2]

4.2 Objectives

Deasy made significant progress in the combination of the base-mediated interconversion of nitroalcohols **82** and the lipase-catalysed resolution. Several limitations were identified and will need to be overcome before the effective combination of the base-catalysed interconversion and the lipase mediated resolution.

The use of DBU is only feasible if the problem of competing chemical acylation could be overcome, such as the use of an alternative acylating agent, or introduction of a solvent to decrease the effective concentration of the acylating agent. Potentially, reduction of the acylating agent loading could also solve this problem.

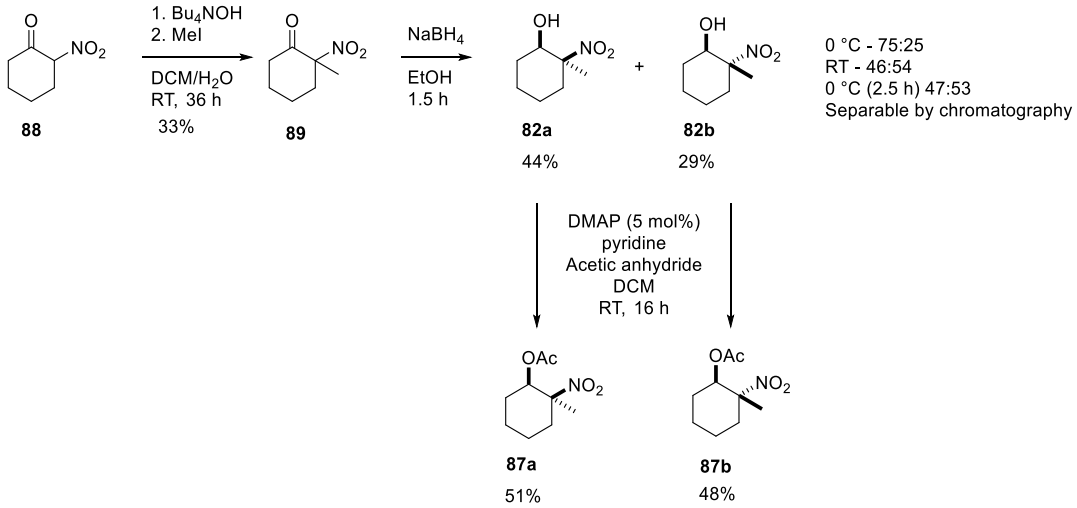
Base-lipase inhibition was a significant limitation during Deasy's work. The simultaneous introduction of the base and the lipase meant that the efficiency of both was reduced. A potential solution is the use of immobilised reagents, or physical separation of the catalysts. CAL-B, an immobilised lipase, gave excellent selectivity in this reaction; the use of an immobilised base could also be beneficial, both for physical separation of the catalyst and potentially for subsequent separation from the reaction mixture.

Further work was warranted to improve the system. As a result, the objectives of this project are:

- Synthesis of the substrate alcohols **82** and the acetates **87**, as reference standards.
- Exploration of a wider range of bases for the interconversion step
- Explore the use of solvents, ideally to find a solvent which works well for both the interconversion step and the resolution step
- Vary the acylating agent, as well as acyl agent loading, as the use of milder acylating agents could overcome the potential problem of chemical acylation
- Vary the reaction conditions
    - Temperature
    - Time
    - Addition time of the reagents
- Explore the use of a flow-like system to physically separate the catalysts and ultimately lead to the high diastereo- and enantioselectivity.

4.3 Synthesis of substrates

The synthesis of the substrates **82a** and **82b** was carried out using a two-step procedure (Scheme 4.7), followed by separation and acylation of the product alcohols to make acetates **87a** and **87b** which are used as standards for HPLC method development.



Scheme 4.7 Synthesis of substrates

The first step was the alkylation of the α-carbon of compound **88** using methyl iodide and tetra-*n*-butyl ammonium hydroxide. This reaction was low yielding, producing two by-products **90** and **91** which were separated by chromatography (Figure 4.8). These products were previously reported by Deasy and were present in roughly the same ratios as previously reported.

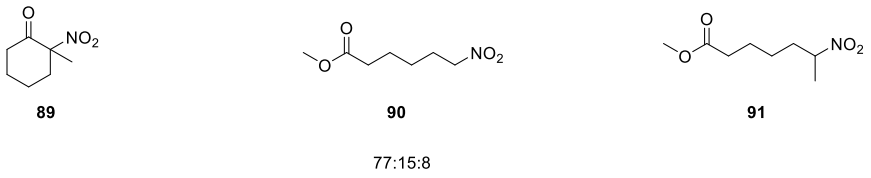
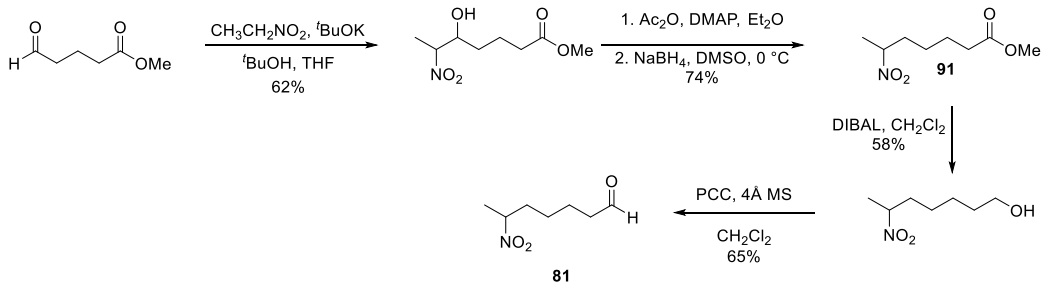


*Figure 4.8 Major products from the alkylation of **88***

The reduction of the ketone **89** was carried out using sodium borohydride as the reducing agent. Increasing the reaction time from 1.5 h to 2.5 h at 0°C gave a similar result to conducting the reaction at room temperature for 1.5 h, giving almost identical product ratios (Scheme 4.7). Separation of diastereomers **82** by chromatography was achieved using 40/60 diethyl ether/hexane as eluent. If 20/80 ethyl acetate/hexane was used as eluent, then the diastereomers did not separate effectively. The alcohol diastereomers were separated and

acylated using DMAP and acetic anhydride, giving the acetates **87a** and **87b** in moderate yields without requiring chromatographic purification.

Deasy previously synthesised aldehyde **81** by a four-step procedure (Scheme 4.8), which furnished compound **91** after the second step. This product was a by-product in the synthesis of ketone **89** and was easily purified by column chromatography and therefore can potentially be utilised in the synthesis of the aldehyde, if required, reducing the waste in the synthesis of **89** and reducing the amount of reactions required in the synthesis of aldehyde **81**.



*Scheme 4.8 Synthesis of methyl-6-nitroheptanal **81***

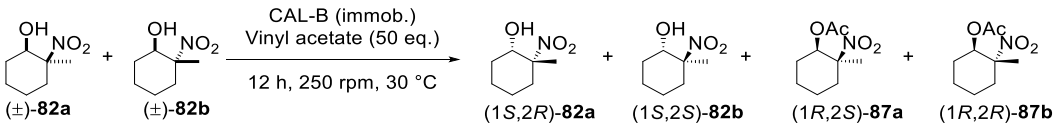
4.4 Initial screens – base, lipase, solvent and acylating agent

Deasy developed a two-pot cycling system, where the reagents were repeatedly exposed to the alternating reaction conditions. Work completed here built on the data collected by Deasy as well as looking at the two reactions individually. A number of experiments were carried out to quantify the effects of different components of the reaction. Considering that one of the best bases for the interconversion was DBU, immobilised DBU was explored as it would enable facile separation from the reaction mixture. Additional bases were explored but with poor results. Similarly, CAL-B (immob) was chosen as the lipase, allowing facile separation.

4.4.1 Solvent screen – towards a one-pot reaction

In order to combine the two separate systems, careful selection of solvent is critical to ensure compatibility with both the base-mediated interconversion, and the lipase-catalysed resolution; it is clear that the use of vinyl acetate as solvent is not feasible due to competing chemical acylation.

Table 4.7 Solvent screen for resolution



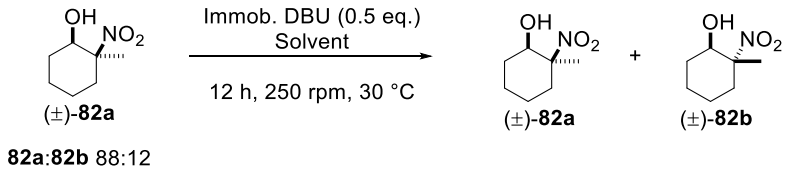
Entry	Solvent	Acetate 87[a,b]	
		cis-87a	*trans*-87b
1	Toluene	4 (>98)	22 (>98)
2	Hexane	9 (>98)	38 (>98)
3	MTBE	10 (>98)	33 (>98)
4	IPA	-[c]	4 (>98)
5	1-Octanol	2 (>98)	11 (33)
6	Ethyl Acetate	2 (>98)	23 (>98)

Entries 1 & 2 were previously carried out by Gavin, and repeated in this work, the results shown are from this work. [a]Relative conversions, determined from *ee* values of the substrate alcohols (ee_s) and product acetates (ee_p), as the presence of some solvents made interpretation of the ^{1}H NMR spectrum difficult; [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [c]no *cis*-**87a** was detected by ^{1}H NMR.

In the solvent screen, we found that the resolution, mediated by CAL-B (immob), performed well in non-polar solvents (toluene and hexane) (Table 4.7, entries 1 and 2) while the interconversion is also conveniently favoured by these non-polar solvents (Table 4.8). Sampling was more reliable using toluene as solvent than using hexane, in which the substrate

was poorly soluble. Interestingly, while the interconversion catalysed by DBU (immob) was sluggish in ethyl acetate (Table 4.8, Entry 6), this solvent provides us with promising diastereomeric discrimination in the resolution step (Table 4.7, Entry 6).

Table 4.8 Solvent screen for the interconversion catalysed by immobilised DBU



(±)-**82a** **82a**:**82b** 88:12 (±)-**82a** (±)-**82b**

Entry	Solvent	Alcohol 82 (%)[a]	
		cis-**82a**	*trans*-**82b**
1	Toluene	68	32
2	Hexane	87	13
3	MTBE	87	13
4	IPA	70	30
5	1-Octanol	-[b]	-[b]
6	Ethyl Acetate	84	16

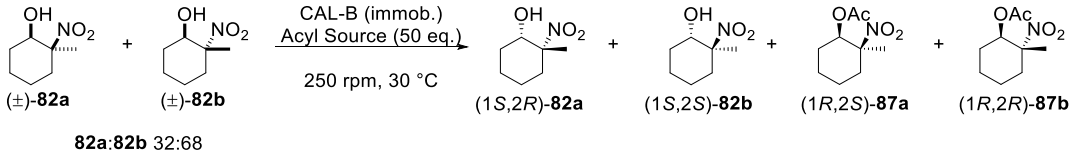
Entries 1 & 2 were previously carried out by Gavin, and repeated in this work, the results shown are from this work. [a]Values determined by ^{1}H NMR; [b]values could not be determined by ^{1}H NMR due to the presence of the solvent.

4.4.2 Variation of acyl source

We briefly explored the use of acylating agents other than vinyl acetate in conjunction with CAL-B (immob). Ramström et al. reported success in a related one-pot reaction using phenyl acetate.[12] Various acylating agents, including ethyl acetate, which could potentially be used as the solvent and the acylating agent, were explored. For these screens, 50 eq. of the acylating agent was used as the acyl source and the solvent; this is less than was used by Deasy in previous work.[1] All acylating agents provides the acetate products *cis*-**87a** and *trans*-**87b** with excellent enantioselectivity, albeit, some with very limited conversion (Table 4.9). Isopropenyl acetate **68**, and isopropyl acetate **80**, both gave similar results, but with conversion <5% for *cis*-**82a** and <15% for *trans*-**82b**, after 12 hours. Ethyl acetate, similarly, showed a low extent of acylation of *cis*-**82a**, but showed high diastereoselectivity (1:8, favouring *trans*-**87b**) when used as the acyl source.

While vinyl acetate and phenyl acetate performed equally well, including over longer reaction times (48 h), use of the higher boiling phenyl acetate did not offer any synthetic advantages over vinyl acetate but was difficult to separate from the product on an analytical scale.

Table 4.9 Variation of acyl source



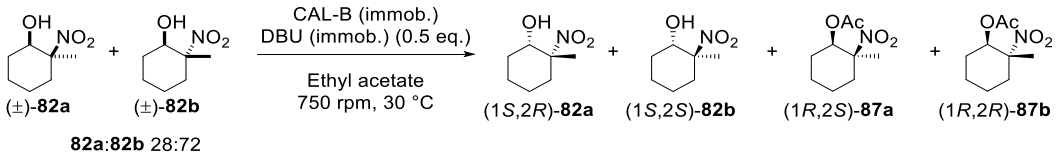
Entry	Acyl Source	Reaction time (h)	Acetate 87 (%)[a,b]	
			cis-87a	*trans*-87b
1	Vinyl Acetate	12	5 (*>98*)	35 (*>98*)
2	Vinyl Acetate	48	12 (*>98*)	46 (*>98*)
3	Isopropenyl Acetate	12	3 (*>98*)	14 (*>98*)
4	Isopropyl Acetate	12	3 (*>98*)	13 (*>98*)
5	Phenyl Acetate	12	6 (*>98*)	34 (*>98*)
6	Phenyl Acetate	48	14 (*>98*)	48 (*>98*)
7	Ethyl Acetate	12	2 (*>98*)	16 (*>98*)

[a]Relative conversions, determined from %*ee* values of the substrate alcohols (ee_s) and product acetates (ee_p); [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

The reaction was carried out again, this time with ethyl acetate as acylating agent, as it is not sufficiently reactive to effect chemical acylation. The reaction time was extended to 120 hours and the agitation was increased to 750 rpm, and extra lipase was added after 48 hours. The acylation proceeded very slowly, giving <10% of the enantiopure acetate *trans*-87b even after extended reaction times (Table 4.10, Entry 1). When using ethyl acetate as solvent the interconversion did not proceed (Table 4.8, Entry 6 and Table 4.10, Entry 2), and when the interconversion and resolution catalysts were added together (Table 4.10, Entry 3), the resolution proceeded enantioselectively. Although the material recovered here was enantioenriched, the extent of both the resolution and the interconversion was not sufficient to be synthetically useful, even after extended reaction times.

These experiments showed that the one-pot reaction was not feasible using ethyl acetate as solvent and DBU (immob) as base, as both the resolution and the interconversion were very inefficient. Although the material recovered here was enantioenriched, the extent of conversion was too low to be useful, even after 5 days, as the interconversion catalysed by DBU (immobilised) was sluggish in the polar solvent. The use of alternative bases in ethyl acetate as solvent was next explored as the diastereoselectivity in the resolution step was promising (Table 4.7, Entry 6).

Table 4.10 One-pot system using ethyl acetate as solvent and acylating agent



(±)-**82a** (±)-**82b** **82a**:**82b** 28:72 (1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

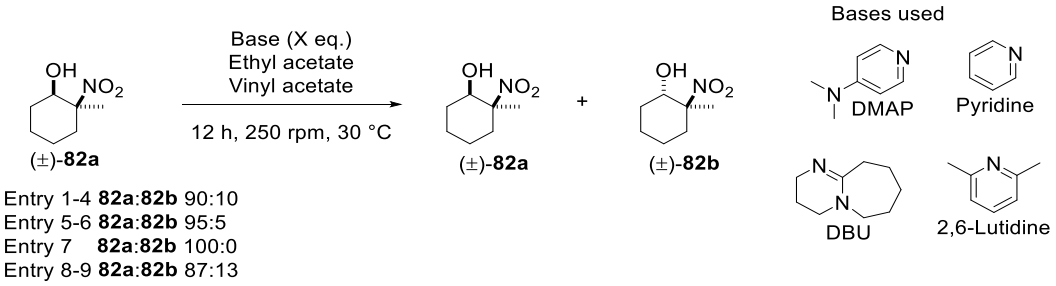
Entry	Reaction Conditions[a]	Time (h)	Alcohol 82		Acetate 87	
			cis-**82a**[b]	*trans*-**82b**[b]	*cis*-**87a**[b]	*trans*-**87b**[b]
1	Lipase, no base[c]	24	29 (3)	65 (12)	0 (>99)	6 (>99)
		120	30 (4)	61 (17)	2 (>99)	7 (>99)
2	Base, no lipase	24	23 (2)	75 (0)	<1 (10)	2 (1)
		120	25 (-)	64 (-)	3 (2)	8 (1)
3	Base & Lipase[c]	24	33 (2)	67 (8)	0 (-)	0 (-)
		120	30 (6)	57 (13)	5 (>99)	9 (>99)

[a]Ethyl acetate added to give [substrate] = 10 mg/mL; reaction run in the absence of base or lipase returned *cis*-**82a**:*trans*-**82b** 28:72 after 120 h i.e. unchanged; [b]% composition of the reaction mixture, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis;[14] [c]extra lipase was added after 48 h.

4.4.3 Exploration of alternative bases using ethyl acetate as solvent

Based on a recent report of the use of immobilised DMAP for the Henry reaction,[15] investigation of DMAP was next explored, as well as structurally related bases (Table 4.11). Ethyl acetate was selected as solvent due to the promising diastereoselectivity in the lipase-mediated resolution (Table 4.7, Entry 6). DMAP performed poorly when only 1 eq. was used, but when the amount of DMAP was increased, up to 10 eq., the interconversion was very successful, outperforming DBU (immob) in this solvent (Table 4.11, Entry 3, 4, 1 and 2, respectively). Most importantly here, with 10 eq. of DMAP, there was no chemical acylation evident even when using 50 eq. of vinyl acetate in ethyl acetate as solvent (Entry 4). The DMAP was removed from the reaction mixture by passing through a short column of silica gel; this was necessary both for clear ^{1}H NMR analysis, and to remove DMAP before chiral HPLC analysis. Use of DMAP (immob) proved to be poorly effective for the interconversion and led to significant background acylation in the presence of vinyl acetate, as well as decreased efficiency of the interconversion (Entry 6). In the presence of CAL-B and vinyl acetate i.e. a one-pot reaction (Entry 7), the acylation proceeded, albeit with very poor enantioselectivity. Interconversion of the diastereomers was not seen when using related bases, lutidine and pyridine (2 eq.), while chemical acylation was evident with lutidine under these conditions (Entry 8 and 9).

Table 4.11 Investigation of different bases using ethyl acetate as solvent



Entry 1-4 **82a**:**82b** 90:10
Entry 5-6 **82a**:**82b** 95:5
Entry 7 **82a**:**82b** 100:0
Entry 8-9 **82a**:**82b** 87:13

Entry	Base	Eq. Base	Eq. Vinyl Acetate	Alcohol 82		Acetate 87	
				cis-**82a**[a]	*trans*-**82b**[a]	*cis*-**87a**[a]	*trans*-**87b**[a]
1	DBU (immob)	1	0	86	14	-	-
			50	80	13	6	1
2		5	0	74	16	-	-
			50	54	22	20	4
3	DMAP	1	0	89	11	-	-
			50	89	11	0	0
4		10	0	0	100	-	-
			50	46	54	0	0
5	DMAP (immob)	1	0	77	23	-	-
			50	89	11	0	0
6		10	0	36	64	-	-
			50	81	10	7	1
7	DMAP & CAL-B (immob)	10	50	71 (2)	ND	22 (7)	6 (4)
8	Pyridine	2	0	87	13	-	-
		2	50	85	15	-	-
9	Lutidine	2	0	87	13	-	-
		2	50	79	18	2	1

Ethyl acetate added to make [substrate] = 10 mg/mL. [a]Numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

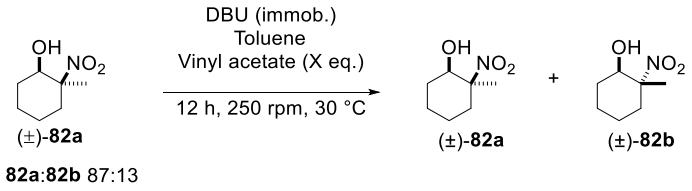
4.4.4 Vinyl acetate loading

As the combination of DBU (immob) and vinyl acetate led to background chemical acylation, this approach was not feasible as it led to reduced enantiopurity of the final product. Predictably, decreasing the concentration of vinyl acetate, as a solution in toluene, significantly reduced the problem of background chemical acylation (5 eq. of vinyl acetate gave <5% chemical acylation after 12 hours) (Table 4.12).

Fortunately, when the loading of vinyl acetate was decreased from 50 eq. to 3 eq. the resolution was equally effective (Scheme 4.9 and Table 4.7, Entry 1 and Table 4.9, Entry 1). This allowed the reduction of the vinyl acetate loading, reducing the extent of competing chemical acylation. It is important to note here that, even though the majority of the starting material used was the *cis* isomer, *cis*-**82a**, the conversion of this to the product was low. The

trans material, *trans*-**82b**, was processed preferentially by the lipase, despite making up only a minority of the material.

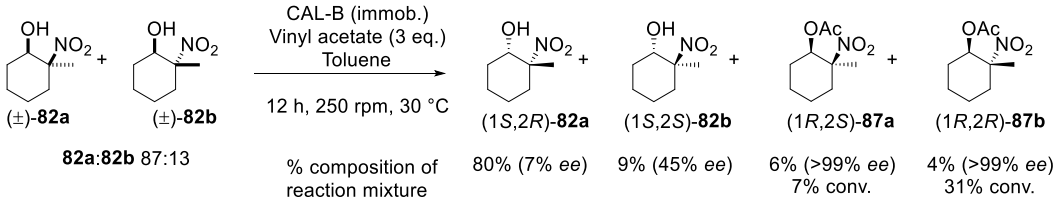
Table 4.12 Investigation of the effect of vinyl acetate loading on interconversion and chemical acylation



82a:**82b** 87:13

Entry	Eq. Vinyl Acetate	Alcohol 82 (%)[a]		Acetate 87 (%)[a]	
		cis-**82a**	*trans*-**82b**	*cis*-**87a**	*trans*-**87b**
1	0	68	32	-	-
2	5	68	28	3	1
3	10	66	28	5	1
4	20	69	24	6	1
5	30	67	22	10	2
6	40	69	20	9	2
7	50	69	20	9	2

[a]% determined by ^{1}H NMR

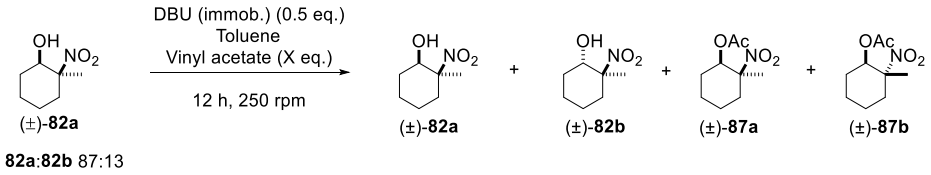


*Scheme 4.9 Resolution of 2-methyl-2-nitrocyclohexanol **82***

4.4.5 Temperature

The next factor explored was the impact of reaction temperature. Both the interconversion and the resolution were carried out separately with varying amounts of vinyl acetate in toluene, at three different temperatures: 30°C, 40°C and 50°C (Table 4.13 and Table 4.14, respectively). The interconversion was much faster at the higher temperatures (Table 4.13). As shown in Table 4.14, the enantioselectivity of the lipase-mediated transesterification of *cis*-**82a** and *trans*-**82b** was unchanged when the temperature was increased from 30°C to 50°C. However, there was a decrease in diastereoselectivity at the higher temperature with a higher proportion of *cis*-**87a** formed at 50°C than at 30°C (Table 4.14).

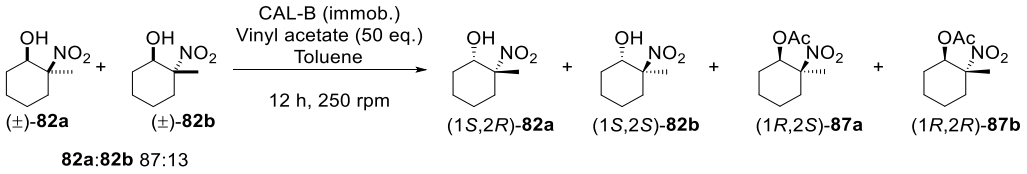
Table 4.13 Temperature effect on the interconversion process



Entry	Eq. VA	30°C[a,b]				40°C[a]				50°C[a]			
		cis-82a	*trans*-82b	*cis*-87a	*trans*-87b	*cis*-82a	*trans*-82b	*cis*-87a	*trans*-82b	*cis*-82a	*trans*-82b	*cis*-87a	*trans*-87b
1	0	68	32	-	-	66	34	-	-	52	48	-	-
2	5	68	28	3	1	70	28	2	1	59	37	3	1
3	10	66	28	5	1	71	25	3	1	49	42	7	2
4	20	69	24	6	1	69	24	6	2	55	33	9	3

[a]% of mixture, determined by ^{1}H NMR by comparison of the areas of the integrals for to relevant alcohols and acetates; [b]data is the same as Table 4.12, Entries 1–4.

Table 4.14 The effect of temperature on resolution



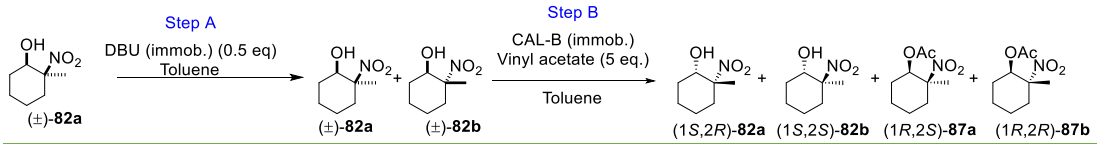
Entry	Eq. Vinyl Acetate	30°C[a,b]		40°C[a,b]		50°C[a,b]	
		cis-87a	*trans*-87b	*cis*-87a	*trans*-87b	*cis*-87a	*trans*-87b
1	5	7 (*>98*)	34 (*>98*)	9 (*>98*)	38 (*>98*)	16 (*>98*)	43 (*>98*)
2	10	9 (*>98*)	38 (*>98*)	14 (*>98*)	43 (*>98*)	16 (*>98*)	40 (*>98*)
3	20	6 (*>98*)	27 (*>98*)	10 (*>98*)	37 (*>98*)	15 (*>98*)	35 (*>98*)

[a]Relative conversions (%), determined by ^{1}H NMR by comparison of the areas of the integrals for to relevant alcohol and acetate; [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

4.5 Towards a one-pot dynamic kinetic resolution

Following this, a series of one-pot and "two-pot" reactions were attempted with the same solvent conditions for both the interconversion and the resolution (Table 4.15).

Table 4.15 A selection of one-pot reactions



Entry	Step	Time (h)	Starting Material 82a:82b	Temp (°C)	Alcohol 82[a]		Acetate 87[a]	
					cis-82a	*trans*-82b	*cis*-87a	*trans*-87b
1	A B	12 12	87:13	50	52 33 *(41)*	48 18 *(87)*	- 17 *(>98)*	- 32 *(>98)*
2[b]	A B	12 12	87:13	50	52 41 *(26)*	48 26 *(80)*	- 11 *(>98)*	- 22 *(>98)*
3	A & B	12	87:13	50	64 *(29)*	9 *(81)*	22 *(>98)*	6 *(>98)*
4	A & B	12	4:96	50	5 *(28)*	46 *(87)*	2 *(>98)*	46 *(>98)*
5	A & B	12	4:96	30	8 *(3)*	79 *(13)*	1 (ND)	12 *(12)*
6	A & B	12	87:13	30	72 *(16)*	9 *(73)*	13 *(>98)*	6 *(>98)*
7	A & B	12	70:30	30	63 *(10)*	18 *(56)*	8 *(>98)*	11 *(>98)*
8	A & B	12	87:13	30	72 *(15)*	9 *(77)*	13 *(>98)*	6 *(>98)*
9[c]	A & B	12	87:13	30	37 *(88)*	8 *(52)*	48 *(>98)*	7 *(>98)*

[a]Given as % composition of the reaction mixture, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [b]entry 2 included a filtration to remove the DBU (immob) before addition of the lipase; [c]entry 9 included molecular sieves.

Entry 1 shows evidence of a dynamic system. The DBU (immob) was not removed prior to CAL-B (immob) addition, and the amount of *trans*-**82b** is less than the amount of *trans*-**87b**. This indicated that the reaction continued past the kinetic limit, also suggesting that the interconversion reaction continued after the addition of the lipase. In the case of Entry 2, the DBU (immob) was removed by filtration before the addition of the CAL-B (immob). If the base

was inactivated by the lipase when they were both present together, then the results for both Entry 1 and Entry 2 should be similar. These experiments showed, for the first time, evidence of a dynamic system when both the base and the lipase were present together, albeit this required a time-delayed addition, key to this system. It also showed that the base and lipase could be present in the reaction mixture at the same time.

The reaction was also carried out where both catalysts were introduced at the same time (Table 4.15). Entries 3 and 4 are identical except for the ratio of starting material. Using predominantly *trans*-**82b** starting material (Entry 4) we saw a much greater conversion, with the conversion essentially at the kinetic limit with some evidence of a dynamic process, as there are equal amounts of *trans*-**82b** and *trans*-**87b** but the % *ee* of *trans*-**82b** is not >98%, as would be expected from a non-dynamic system.

The one-pot procedure, where both the catalysts were added in at the same time, was repeated at lower temperature, 30°C, using *trans*-**82b** and *cis*-**82a** enriched material (Entries 5-7). The conversions in this case were poorer compared to the higher temperature but still retained high stereoselectivity, as expected.

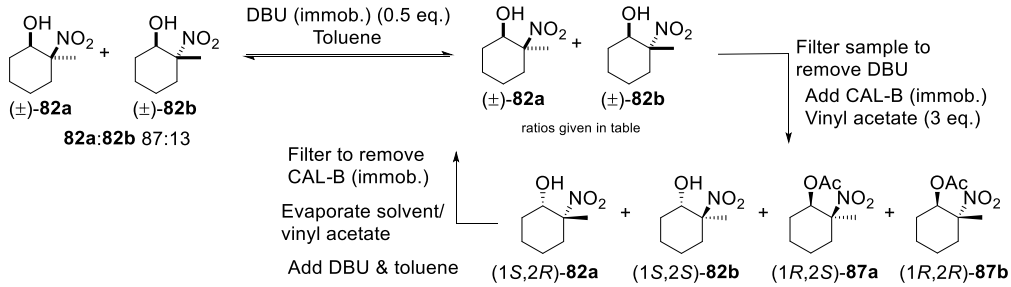
The effect of water was also investigated at this temperature (Entries 8 and 9). The reaction was run with (Entry 9) or without (Entry 8) molecular sieves, again with both the catalysts introduced at the same time. Interestingly, the presence of molecular sieves reversed the diastereoselectivity of the reaction, with the *cis*-**87a** diastereomer being preferred here, but no change in the interconversion efficiency, showing that the presence of small amounts of water is not affecting the DBU (immob) efficiency.

As the time delayed addition of the catalysts was shown to be effective, the cycling of the material through the two different reaction conditions consecutively was investigated.

In the first reaction cycle (Table 4.16, Entry 1), the material was cycled through the two steps, removing one catalyst prior to addition of the next by a simple filtration through a plug of Celite® and magnesium sulfate, and removal of the vinyl acetate by rotary evaporation at the end of the resolution step, before addition of toluene and DBU (immob) to start the cycle again. Here, the equivalents of vinyl acetate were reduced to 3, to minimise the amount of chemical acylation. For the initial cycles, the first procedure (Entry 1) performed both the interconversion and the resolution at 50°C. While this system reached the kinetic limit after

two cycles, (*trans*-**82b**:*trans*-**87b** = 1:1), there was evidence of a dynamic system i.e. that *trans*-**82b** was being interconverted albeit slowly (76% *ee*, >98% *ee*, respectively). The diastereoselectivity was poor, giving equal amounts of the acetates *cis*-**87a** and *trans*-**87b** at the end of the third cycle, with, overall, a poor conversion for a dynamic system (acetate **87** making up just over 50% of the mixture). It was assumed that the elevated temperature was the major cause of the reduced diastereoselectivity, as observed previously (Table 4.14).

Table 4.16 One-pot reaction – removal of each catalyst before addition of the next



Entry	Step	Temperature (°C)	Alcohol 82[a]		Acetate 87[a]	
			cis-**82a**	*trans*-**82b**	*cis*-**87a**	*trans*-**87b**
1	1A	All steps carried out at 50°C	56 (-)	44 (-)	0 (-)	0 (-)
	1B		56 (23)	17 (58)	13 (>98)	14 (>98)
	2A		35 (18)	34 (22)	15 (>98)	16 (>98)
	2B		30 (42)	25 (76)	20 (>98)	25 (>98)
	3A		23 (30)	25 (40)	23 (>98)	29 (>98)
	3B		21 (79)	23 (>98)	26 (>98)	30 (>98)
2	1A	50	34 (4)	66 (5)	0 (-)	0 (-)
	1B	30	38 (10)	42 (31)	4 (>98)	16 (>98)
	2A	50	28 (4)	41 (16)	6 (>98)	25 (>98)
	2B	30	25 (14)	27 (70)	8 (>98)	40 (>98)
	3A	50	20 (8)	24 (2)	10 (>98)	47 (>98)
	3B	30	28 (14)	13 (66)	11 (>98)	47 (>98)
3[b]	1A	50	36 (1)	64 (3)	0 (-)	0 (-)
	1B	30	36 (9)	39 (46)	4 (>98)	20 (>98)
	2A	50	27 (6)	32 (33)	8 (>98)	33 (97)
	2B	30	25 (20)	25 (78)	10 (>98)	40 (>98)
	3A	50	25 (21)	17 (70)	13 (>98)	45 (96)
	3B	30	25 (24)	19 (78)	12 (>98)	43 (97)

Step A, interconversion: DBU (immob) (0.5 eq.), toluene (10mg/mL substrate). Step B, resolution: CAL-B (immob), vinyl acetate (3 eq.), toluene (1mL/10mg). Each step was carried out for 12 hours, except step 1A for Entry 2 & Entry 3 which were 18 h. [a]Expressed as a % determined by integration of the relevant ^{1}H NMR signals, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [b]evaporation step was omitted

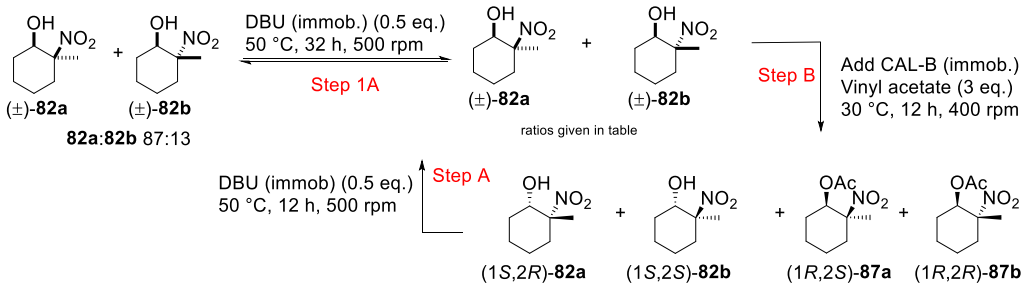
Following this, the same cycling system was maintained, this time with the resolution being carried out at the lower temperature of 30°C (Table 4.16, Entry 2), and the initial interconversion step being extended from 12 hours to 18 hours, which gave a greater

proportion of *trans*-**82b** present for the first resolution step (34:66, compared to 56:44 after 12 h). Again, before the addition of each catalyst, the previous catalyst was removed by filtration, and the vinyl acetate was removed by rotary evaporation at the end of the cycle. Throughout three cycles the enantioselectivity remained consistently high, with enantiopure *cis*-**87a** and *trans*-**87b** observed by HPLC analysis. In this case, acetate **87** again made up over 50% of the reaction mixture, but the product *trans*-**87b** was strongly preferred (47% *trans*-**87b**, 11% *cis*-**87a**, >98% *ee* for both). Here, there was no evidence that the reaction was complete (alcohol **82b** << 98% *ee*), but when it was continued through another cycle the product started to hydrolyse. The presence of a removable impurity made the spectra more difficult to interpret; it was shown that the impurity could be removed by chromatography on silica gel, and that it arose from the immobilised lipase. Here, it was shown that the resolution proceeded better at the lower temperature, and thus, it was decided to continue under these conditions.

As a result of the sustained excellent enantioselectivity, the reaction was carried out again, this time removing the evaporation step, resulting in the DBU (immob) being exposed to vinyl acetate but giving an overall simpler process from a work-up point of view (Table 4.16, Entry 3). Only a modest decrease in enantiopurity was observed, suggesting that the removal of the evaporation step was well tolerated. At the end of 3 cycles the ^{1}H NMR and HPLC showed 43% of the reaction mixture made up of the acetate *trans*-**87b** (97% *ee*), and 12% acetate *cis*-**87a** (>98% *ee*). While this is lower than when the evaporation step is included, it is not significantly lower.

Encouraged by the success of the “two-pot” procedures, as well as previous evidence that the interconversion continued after the addition of CAL-B (immob), we decided to attempt a series of sequential one-pot reactions, where both catalysts were present at the same time, albeit after a staggered addition. In these reactions, the DBU (immob) was added, reacted for 32 hours, at 50°C, followed by a decrease in temperature, and the addition of CAL-B (immob) and vinyl acetate.

Table 4.17 One-pot reactions



Entry	Step	Notes	Alcohol 82[a]		Acetate 87[a]	
			cis-82a	*trans*-82b	*cis*-87a	*trans*-87b
1	1A	Filter to remove catalyst after step B, no evaporation step.	36 *(4)*	64 *(0)*	0 *(-)*	0 *(-)*
	1B		20 *(28)*	26 *(96)*	14 *(>98)*	41 *(>98)*
	2A		16 *(13)*	26 *(32)*	15 *(94)*	42 *(>98)*
	2B		18 *(28)*	22 *(85)*	15 *(96)*	46 *(97)*
	3A		18 *(24)*	14 *(62)*	20 *(96)*	48 *(96)*
	3B		19 *(39)*	19 *(95)*	19 *(95)*	42 *(90)*
2	1A	Filter & evaporation after step B	36 *(4)*	64 *(0)*	0 *(-)*	0 *(-)*
	1B		18 *(39)*	27 *(>98)*	14 *(>98)*	41 *(>98)*
	2A		16 *(16)*	27 *(7)*	17 *(92)*	40 *(97)*
	2B		17 *(16)*	25 *(62)*	16 *(97)*	43 *(98)*
	3A		9 *(7)*	21 *(13)*	19 *(94)*	51 *(97)*
	3B		11 *(55)*	17 *(87)*	18 *(97)*	53 *(96)*
3	1A	No filtration or evaporation step carried out, no additional catalyst added	33 *(7)*	67 *(0)*	0 *(-)*	0 *(-)*
	1B		20 *(41)*	25 *(93)*	16 *(94)*	40 *(>98)*
	2A		17 *(89)*	30 *(>98)*	19 *(98)*	34 *(98)*
	2B		26 *(80)*	34 *(55)*	17 *(98)*	23 *(97)*
	3A		23 *(57)*	44 *(37)*	15 *(96)*	17 *(96)*
	3B		26 *(52)*	45 *(31)*	15 *(96)*	15 *(95)*
4	1A	No filtration or evaporation step.	33 *(4)*	67 *(1)*	0 *(-)*	0 *(-)*
	1B		19 *(35)*	26 *(>98)*	14 *(>98)*	41 *(>98)*
	2A		19 *(71)*	43 *(36)*	18 *(98)*	20 *(>98)*
	2B		20 *(64)*	41 *(38)*	17 *(98)*	22 *(>98)*
	3A		28 *(32)*	53 *(11)*	13 *(95)*	6 *(85)*
	3B		28 *(31)*	48 *(17)*	13 *(94)*	11 *(63)*

Step A, interconversion: DBU (immob) (0.5 eq.), toluene (1mL/10mg substrate), 50°C, 500 rpm. Step B, resolution: CAL-B (immob), vinyl acetate (3 eq.), toluene (1mL/10mg), 30°C, 400 rpm. Each step was carried out for 12 hours, except step 1A for which was 32 h. [a]Expressed as a % determined by integrating of the relevant ^{1}H NMR signals. Numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.

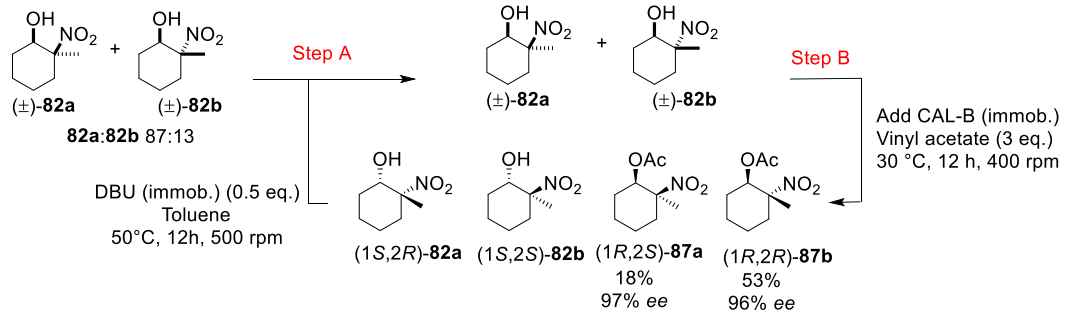
When no evaporation step was carried out (Table 4.17, Entry 1) the overall enantiopurity of the products *cis*-**87a** and *trans*-**87b** were reduced (95% *ee*, 90% *ee*, respectively), as well as reduced conversion overall. When both the filtration and the evaporation step were removed, the overall amount of acetate **87** after 3 cycles was reduced (Table 4.17, Entry 4). In this case, extra catalyst was added to the reaction mixture after each step, but without the removal of the previous catalysts, it gave a similar result to when no additional catalyst was added (Entry 3). This suggests that the presence of the old catalyst is detrimental to the reaction, resulting in hydrolysis of the product acetates *cis*-**87a** and *trans*-**87b** during each racemisation step, as well as evidence of chemical acylation, for both Entry 3 and 4. Although the presence of the catalysts is tolerated through one reaction cycle, removal of the old catalysts is essential before starting the cycle again.

Table 4.17, entry 2 shows the optimised one-pot procedure. It was found that the addition of the catalysts needed to be staggered, as DBU (immob) is less effective in the presence of CAL-B (immob). The DBU (immob) was added first, in this case interconversion was carried out for 32 h at 50°C for step 1A. Although it was subsequently shown that 32 hours was not required and that the same *cis*-**82a**:*trans*-**82b** ratio (~36:64) was reached after only 18 hours. Critically, due to staggered addition of reagents, it was possible to conduct the interconversion at 50°C and then cool the reaction mixture to 30°C for the lipase-mediated resolution, carried out in the same reaction vessel, with the vinyl acetate and the CAL-B (immob) added after the interconversion and cooling to 30°C.

The starting material used was a mixture of *cis*-**82a** and *trans*-**82b** (87:13) instead of aldehyde **81**. In the presence of DBU only, aldehyde **81** will cyclise, and in this case, the mixture of nitroalcohols **82** or aldehyde **81** would be expected to give the same outcome under the conditions for step 1A.

These results are promising, showing, for the first time, a one-pot, dynamic system for the resolution of alcohol **82**. While this is not the desired one-pot reaction, it represents a significant move towards a one-pot procedure (Scheme 4.10). In order to achieve this, several parameters were examined, and their role evaluated. Filtration (after step B) is necessary to remove the immobilised catalysts but does not need to be carried out after each step (A and B), only the end of each cycle (after step B). Sequential addition of the catalysts was found to be key to this one-pot procedure, where the immobilised base was added to the reaction mixture first, to allow interconversion to be carried out, followed by the introduction of the

immobilised lipase, to carry out the resolution. While the evaporation step (after B) was shown to give better enantiopurity after three cycles as well as more product (~60% vs ~50%) than without the evaporation step, it could be removed if necessary to simplify the procedure.



Scheme 4.10 Optimised conditions for the cycling system

The final ratio of the products and substrates show, that, although not a "traditional" one-pot reaction, a dynamic system has been engineered, through extensive optimisation of the individual steps.

4.6 Conclusion

Significant progress has been made in the individual elements of the dynamic resolution process for *trans*-**87b**. Efficient kinetic resolution has been effected for both the *trans*-2-methyl-2-nitrocyclohexanol **82b** and *cis*-2-methyl-2-nitrocyclohexanol **82a**. Significantly, CAL-B (immob) displayed high diastereoselectivity, selectively acetylating *trans*-**82b** efficiently and with excellent enantioselectivity.

The conditions were further explored, focusing on conditions which work for the individual parts. Conditions were developed which furnished a dynamic system albeit with poor efficiency. Key to this was the timing of the reagent addition, by first adding the base, to carry out the interconversion, then the lipase for the resolution, we successfully demonstrated a dynamic system was possible.

In conclusion, achieving DKR in the intramolecular nitroaldol reaction is extremely challenging due to the number of competing processes arising. By careful exploration of the process conditions including variation of biocatalyst, base and solvent, as well as reducing the loading of the acylating agent, we have demonstrated for the first time the feasibility of this process. Key to this system was the time delayed addition of the catalysts, which not only avoided base-lipase inhibition, but also allowed the reaction halves to be carried out at different temperatures.

Although extensive work has been carried out to demonstrate the utility of this dynamic kinetic resolution, further work, to extend the substrate scope was not carried out, as the synthetic utility of this protocol was not sufficient to justify such research.

4.7 References

1. Deasy, R. E. Ph.D. Thesis, NUI Cork, 2012.
2. Foley, A. M.; Gavin, D. P.; Deasy, R. E.; Milner, S. E.; Moody, T. S.; Eccles, K. S.; Lawrence, S. E.; Maguire, A. R. *Tetrahedron* 2018; *74*:1435-43.
3. Henry, L. *C. R. Acad. Sci. Ser. C.* 1895; *120*:1265-8.
4. Feuer, H. *The chemistry of the nitro and nitroso groups*; Interscience Publishers, 1970.
5. Luzzio, F. A. *Tetrahedron* 2001; *57*:915-45.
6. Marcelli, T.; van der Haas, R. N.; van Maarseveen, J. H.; Hiemstra, H. *Angew. Chem. Int. Ed.* 2006; *45*:929-31.
7. Palomo, C.; Oiarbide, M.; Laso, A. *Eur. J. Org. Chem.* 2007; *2007*:2561-74.
8. Milner, S. E.; Moody, T. S.; Maguire, A. R. *Eur. J. Org. Chem.* 2012; *2012*:3059-67.
9. Purkarthofer, T.; Gruber, K.; Gruber-Khadjawi, M.; Waich, K.; Skranc, W.; Mink, D.; Griengl, H. *Angew. Chem. Int. Ed.* 2006; *45*:3454-6.
10. Kitayama, T.; Rokutanzono, T.; Nagao, R.; Kubo, Y.; Takatani, M.; Nakamura, K.; Okamoto, T. *J. Mol. Catal. B: Enzym.* 1999; *7*:291-7.
11. Vongvilai, P.; Angelin, M.; Larsson, R.; Ramström, O. *Angew. Chem. Int. Ed.* 2007; *46*:948-50.
12. Vongvilai, P.; Larsson, R.; Ramström, O. *Adv. Synth. Catal.* 2008; *350*:448-52.
13. Milner, S. E.; Brossat, M.; Moody, T. S.; Elcoate, C. J.; Lawrence, S. E.; Maguire, A. R. *Tetrahedron: Asymmetry* 2010; *21*:1011-6.
14. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* 1982; *104*:7294-9.
15. Das, D.; Pathak, G.; Rokhum, L. *RSC Adv.* 2016; *6*:104154-63.

Experimental

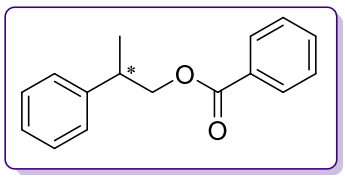
Contents

6.1 General procedures

Solvents were distilled prior to use as follows: dichloromethane (DCM) was distilled from phosphorus pentoxide, ethyl acetate was distilled from potassium carbonate, THF and toluene were distilled from sodium and benzophenone. Hexane was distilled prior to use. Molecular sieves were activated by heating at 150 °C overnight. Organic phases were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate. Infrared spectra were recorded neat using a Perkin Elmer FTIR UATR2 spectrometer. ^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer, and all spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ_H & δ_C) are reported in parts per million (ppm) and coupling constants are expressed in Hertz (Hz). Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer in electrospray ionisation (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionisation mode (ESI) using 50% water/acetonitrile containing 0.1% formic acid as eluent. Flash chromatography was performed using Kieselgel Silica Gel 60, 0.040–0.063 mm (Merck). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254). Visualisation was achieved by UV (254 nm) light detection and $KMnO_4$ staining. Optical rotation was measured on a Perkin-Elmer 141 polarimeter at 589 nm in a 1 cm cell; concentrations (c) are expressed in g/100 mL. $[\alpha]_D^{20}$ is the specific rotation and is expressed in units of 10^{-1} deg cm^2 g^{-1}. Hydrolases were from Almac Sciences except Amano PS Lipase, CAL-B (on ImmoBead 150), Hog pancreas lipase, *Pseudomonas fluorescens* (immobilised on Sol-gel), and Lipase from *Candida cylindracea* which were purchased from Sigma Aldrich chemical company. Transaminases were obtained as whole cell preparations from BIOMERIT in UCC. All biotransformations were performed on a VWR Incubating Mini Shaker 4450. All reagents are analytical grade and purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, Fluorochem or TCI. HPLC analysis was performed on a Waters alliance 2690 separations module with a PDA detector. Enantiomeric excess values were measured determined using Chiralcel® chiral columns (5 × 250 mm) purchased from Daicel Chemical Industries, Japan or Phenomenex Cellulose and Amylose columns (5 × 250 mm), purchased from Phenomenex Inc., UK. Mobile phase and flow rate, detector wavelength and column temperature are included where appropriate.

6.2 Towards the lipase-mediated resolution of 2-phenyl-1-propanol **1**

2-Phenylpropyl benzoate **21**[1]

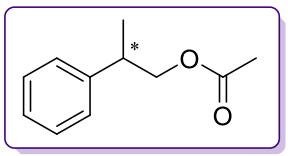


Benzoyl chloride (3.960 g, 28.2 mmol) in DCM (20 mL) was added slowly to a stirring solution of 2-phenyl-1-propanol **1** (4.0 mL, 3.98 g, 29.2 mmol) and triethylamine (4.67 mL, 3.40 g, 33.6 mmol) in DCM (20 mL) at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with DCM (90 mL). The solution was washed with water (2 × 100 mL), aq. HCl (10%, 2 × 100 mL), brine (150 mL), dried, filtered and concentrated to give the crude product **21** as a pale yellow oil. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent, giving the pure ester **21** (4.434 g, 66%) as a colourless oil.

ν_{max} (ATR) 2969 (C–H), 1716 (C=O), 1268, 1110 (C–O), 698 (C–H Ar) cm^{-1}; δ_H (300 MHz) 1.40 (d, 3H, *J* = 7.0, CH_3), 3.17–3.33 (sym m, 1H, CH), 4.33–4.48 (sym m, 2H, C(1)H_2), 7.19–7.46 (m, 7H, ArH), 7.49–7.57 (m, 1H, ArH), 7.94–8.04 (m, 2H, ArH) ppm; δ_C (75 MHz) 18.1 (CH_3), 39.1 (CH), 69.9 (CH_2), 126.7, 127.4, 128.3, 128.5, 129.6 (5 × aromatic CH), 130.4 (aromatic C), 132.9 (aromatic CH), 143.2 (aromatic C), 166.5 (C=O) ppm; HRMS (ES^+): $[M+H]^+$ 241.1229 (calculated: 241.1223); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL min^{-1}], R_t = 10.0 min *(R)*-**21**, 12.4 min *(S)*-**21**.

All data are in agreement with previously reported data except signal for C(1)H_2 is reported as a doublet, and the signal here is a symmetrical multiplet.

2-Phenylpropyl acetate **22**[2]



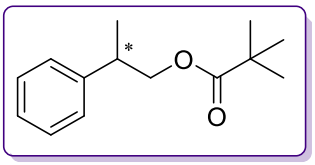
Anhydrous pyridine (2.7 mL, 2.61 g, 33.0 mmol, 1.5 eq.) and acetyl chloride (1.6 mL, 1.73 g, 22.0 mmol, 1.0 eq.) were sequentially added to a stirring solution of 2-phenyl-1-propanol **1** (3.0 g, 22.0 mmol, 1.0 eq.) and DMAP (134 mg, 1.1 mmol, 5 mol%) in DCM (75 mL) at 0 °C. The solution was removed from ice bath and was stirred for 24 h at room temperature. The reaction was quenched with sat. aq. $NaHCO_3$ (40 mL) and was stirred until no more gas was evolved (approx. 40 mins). The layers were separated, and the organic layer was washed with sat. aq. $CuSO_4$ (3 × 40 mL). The combined aqueous layers were extracted

with DCM (50 mL). The organic layers were combined and washed with water (2 × 100 mL), sat. aq. $NaHCO_3$ (100 mL), brine (100 mL), dried, filtered and concentrated to give the crude product as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **22** (2.16 g, 55%) as a colourless oil.

ν_{max} (ATR) 2968 (C–H), 1737 (C=O), 1373, 1227 (C–O), 699 (C–H Ar) cm^{-1}; δ_H (300 MHz) 1.30 (d, 3H, *J* = 7.1, CH_3), 2.00 (s, 3H, $COCH_3$), 3.00–3.19 (sym m, 1H, CH), 4.05–4.27 (sym m, 2H, CH_2), 7.11–7.41 (m, 5H, ArH) ppm; δ_C (75 MHz) 18.1 (CH_3), 20.9 ($CO\mathbf{C}H_3$), 38.9 (CH), 69.4 (CH_2), 126.7, 128.5, 127.3 (3 × aromatic CH), 143.2 (aromatic C), 171.0 (C=O) ppm; enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99.5/0.5, flow rate = 0.5 mL min^{-1}], R_t = 30.1 min *(S)*-**22**, 34.5 min *(R)*-**22**.

Data is in agreement with previous reports.[3]

2-Phenylpropyl pivalate **23**

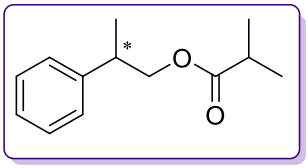


Prepared following the procedure for **22** using anhydrous pyridine (2.7 mL, 2.61 g, 33.0 mmol, 1.5 eq.), pivaloyl chloride (2.7 mL, 22.0 mmol, 1.0 eq.), 2-phenyl-1-propanol **1** (3.04 g, 22.0 mmol, 1.0 eq.) and DMAP (134 mg, 1.1 mmol, 5 mol%) in DCM (75 mL) to give the crude product **23** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **23** (3.273 g, 68%) as a colourless oil.

ν_{max} (ATR) 2971 (C–H), 1727 (C=O), 1282, 1150 (C–O), 699 (C–H) cm^{-1}; δ_H (300 MHz) 1.13 [9H, s, $C(CH_3)_3$], 1.31 (d, 3H, *J* = 6.9, CH_3), 3.02–3.29 (sym m, 1H, CH), 4.15 (qd, 2H, *J* = 10.8, 6.9, CH_2), 7.15–7.37 (m, 5H, ArH) ppm; δ_C (75 MHz) 16.9 (CH_3), 26.1 [$C(\mathbf{C}H_3)_3$], 37.7 [$\mathbf{C}(CH_3)_3$], 38.0 (CH), 68.2 (CH_2), 125.6, 126.3, 127.4 (3 × aromatic CH), 142.3 (aromatic C), 177.3 (C=O) ppm; HRMS (ES^+): $[M+H]^+$ 221.1541 (calculated: 221.1542); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 0.5 mL min^{-1}], R_t = 9.5 min *(S)*-**23**, 13.1 min *(R)*-**23**.

This compound is novel and has been fully characterized in this investigation.

2-Phenylpropyl isobutyrate **24**



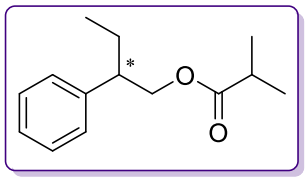
Prepared following the procedure for **22** using anhydrous pyridine (1.05 mL, 0.98 g, 15.0 mmol, 1.5 eq.), isobutyryl chloride (1.2 mL, 10.0 mmol, 1.0 eq.), 2-phenyl-1-propanol **1** (1.4 mL, 10

mmol, 1.0 eq.) and DMAP (61 mg, 0.5 mmol, 5 mol%) in DCM (35 mL) to give the crude product **24** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (90/10) as eluent gave pure ester **24** (1.862 g, 90%) as a colourless oil.

ν_{max} (ATR) 2972 (C–H), 1732 (C=O), 1190, 1153 (C–O), 699 (C–H Ar) cm^{-1}; δ_H (300 MHz) 1.09 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.11 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.31 [d, 3H, *J* = 7.0, C(4)**H**$_3$], 2.50 (sept, 1H, *J* = 7.0, COCH), 3.00–3.22 (sym m, 1H, C**H**CH$_2$), 4.00–4.32 (sym m, 2H, C(1)**H**$_2$), 7.10–7.43 (m, 5H, ArH) ppm; δ_C (75 MHz) 18.0 [C(4)H$_3$], 18.9 [CH(**C**H$_3$)$_2$], 34.0 [**C**H(CH$_3$)$_2$], 39.0 (**C**HCH$_2$), 69.1 (CH$_2$), 126.6, 127.3, 128.4 (3 × aromatic CH), 143.3 (aromatic C), 177.0 (C=O) ppm; HRMS (ES^+): $[M+H]^+$ 207.1381 (calculated: 207.1380); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL min^{-1}], R_t = 6.6 min *(R)*-**24**, 11.1 min *(S)*-**24**.

This compound is novel and has been fully characterized in this investigation.

2-Phenylbutyl isobutyrate **25**

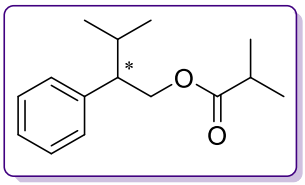


Prepared following the procedure for **22** using anhydrous pyridine (0.49 mL, 0.48 g, 6.1 mmol, 1.5 eq.), isobutyryl chloride (0.42 mL, 4.0 mmol, 1.0 eq.) 2-phenyl-1-butanol **3** (0.6 mL, 3.9 mmol, 1.0 eq.), and DMAP (27 mg, 0.2 mmol, 5 mol%) in DCM (22 mL) to give the crude product **25** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **25** (0.428 g, 50%) as a colourless oil.

ν_{max} (ATR) 2969 (C–H), 1732 (C=O), 1190, 1153 (C–O), 699 (C–H Ar) cm^{-1}; δ_H (300 MHz) 0.83 (t, 3H, *J* = 7.4, CH$_2$C**H**$_3$), 1.06 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.09 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.52–1.71 [m, 2H, C**H**$_2$CH$_3$], 2.47 [sept, 1H, *J* = 7.0, C**H**(CH$_3$)$_2$], 2.75–2.92 (sym m, 1H, C**H**CH$_2$O), 4.21 (d, 2H, *J* = 7.0, C**H**$_2$O), 7.00–7.48 (m, 5H, ArH) ppm; δ_C (75 MHz) 11.8 [C(4)H$_3$], 18.9 [CH(**C**H$_3$)$_2$], 25.3 [C(3)H$_2$], 34.0 [**C**H(CH$_3$)$_2$], 46.8 [C(2)H], 68.0 (CH$_2$O), 126.6, 128.0, 128.4 (3 × aromatic CH), 141.9 (aromatic C), 177.1 (C=O) ppm; HRMS (ES^+): $[M+H]^+$ 221.1537 (calculated: 221.1542); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98/2, flow rate = 1 mL min^{-1}], R_t = 6.1 min, 8.3 min.

This compound is novel and has been fully characterized in this investigation.

3-Methyl-2-phenylbutyl isobutyrate **26**

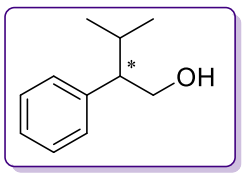


Prepared following the procedure for **22** using anhydrous pyridine (0.31 mL, 0.304 g, 3.8 mmol, 1.5 eq.), isobutyryl chloride (0.27 mL, 0.28 g, 2.6 mmol, 1.0 eq.), 3-methyl-2-phenyl-1-butanol **3** (0.413 g, 2.5 mmol, 1.0 eq.) and DMAP (16 mg, 0.13 mmol, 5 mol%) in DCM (10 mL) to give the crude product **26** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **26** (0.212 g, 35%) as a colourless oil.

ν_{max} (ATR) 2969 (C–H), 1732 (C=O), 1190, 1154 (C–O), 700 (C–H Ar) cm^{-1}; δ_H (300 MHz) 0.76 [d, 3H, *J* = 6.7, C(4)H_3], 0.89–1.10 [m, 9H, C(4)H_3 & CH(C**H**$_3$)$_2$], 1.88–2.10 [m, 1H, CH_2C**H**$(CH_3)_2$], 2.41 (sept, 1H, *J* = 7.0, COCH), 2.57–2.79 (sym m, 1H, C**H**CH_2), 4.35 (d, 2H, *J* = 6.8, CH_2), 7.00–7.48 (m, 5H, ArH) ppm; δ_C (75 MHz) 18.8, 20.7, 20.9 (3 × CH_3 signals for 4 × CH_3), 30.4 [C(3)H], 34.0 (CO**C**H), 51.8 [C(2)H], 66.2 (CH_2), 126.4, 128.1, 128.5 (3 × aromatic CH), 141.6 (aromatic C), 177.1 (C=O) ppm; HRMS (ES^+): $[M+H]^+$ 235.1687 (calculated: 235.1698); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL min^{-1}], R_t = 6.0 min, 8.4 min.

This compound is novel and has been fully characterized in this investigation.

3-Methyl-2-phenylbutan-1-ol **3**[4]



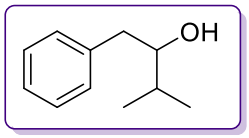
Grignard reaction:[5] In a flame dried 2-neck flask equipped with a reflux condenser, magnesium (1.505 g, 61.9 mmol) and a crystal iodine were vigorously stirred in diethyl ether (5 mL) for 1.5 h under a blanket of nitrogen. To this mixture isopropylbromide **45** (5.15 mL, 6.747 g, 54.9 mmol) in diethyl ether (15 mL) was added slowly to maintain a gentle reflux. On completion of the addition the solution was heated to reflux. After 40 mins, the solution was cooled to 0 °C using an ice bath, and a solution of styrene oxide **44** (5.72 mL, 6.006 g, 50.0 mmol) in diethyl ether (15 mL) was added over 25 mins and the solution was allowed to warm to room temperature and stirred for 40 mins, after which TLC showed the disappearance of styrene oxide **44**. The reaction solution was poured onto ice-water mixture (35 mL) and acidified to pH = 1 using conc. H_2SO_4. The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 20 mL). The organic layers were combined and washed with sat. aq. Na_2CO_3 (2 × 20 mL), water (3 × 20 mL), brine (40 mL), dried, filtered and concentrated to give the crude product as a yellow oil (5.911 g). Purification by column chromatography on silica

gel using hexane/ethyl acetate (90/10) gave the product **3** as a yellow oil (1.70 g, 21%, R_f = 0.1).

ν_{max} (ATR) 3368 (OH), 2957 (C–H), 1494, 1453, 1366, 1056, 1031, 699 (C–H) cm^{-1}; δ_H (300 MHz) 0.74 [d, 3H, *J* = 6.9, C(4)H_3], 0.99 [d, 3H, *J* = 6.9, C(4)H_3], 1.82–2.03 [m, 1H, C(3)H], 2.51 [td, 1H, *J* = 8.7, 5.0, C(2)H_2], 3.72–4.02 (m, 2H, CH_2), 7.16–7.37 (m, 5H, ArH) ppm; δ_C (75 MHz) 21.0 (2 × CH_3), 30.1 [C(3)H], 55.8 [C(2)H], 65.2 [C(1)H_2], 126.7, 128.5, 128.7 (3 × aromatic CH), 141.7 (aromatic C) ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL min^{-1}], R_t = 21.9 min, 28.2 min.

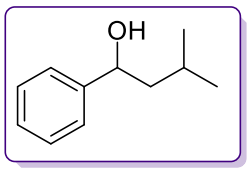
All data is in agreement with previously reported data.[4]

Another product was also isolated from the column (1.687 g, 21%, R_f = 0.2) and was identified as the Meinwald rearrangement product, 3-methyl-1-phenylbutan-2-ol **47**, and agrees with previously reported data for this compound.[6]



ν_{max} (ATR) 3408 (OH), 2956 (C–H), 1495, 1453, 1367, 1075, 1031, 698 (C–H) cm^{-1}; δ_H (300 MHz) 1.00 [d, 6H, *J* = 6.8, CH(C**H**$_3$)$_2$], 1.69–1.85 [sym m, 1H, C**H**$(CH_3)_2$], 2.59 (dd, 1H, *J* = 13.6, 9.5, one of CH_2Ph), 2.85 (dd, 1H, *J* = 13.6, 3.0, one of CH_2Ph), 3.48–3.68 (m, 1H, C**H**OH), 7.03–7.50 (m, 5H, ArH) ppm; δ_C (75 MHz) 17.7, 19.4 (2 × CH_3), 33.1 (**C**HCH_3), 40.9 (**C**H_2Ph), 77.6 (**C**HOH), 125.9, 127.3, 128.6, (3 × aromatic CH), 143.6 (aromatic C) ppm.

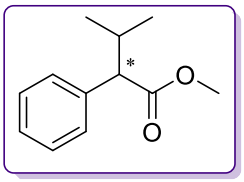
3-Methyl-1-phenylbutan-1-ol **46**



Magnesium (1.46 g, 60.0 mmol) and iodine (1 crystal) were stirred at high speed with small amount of diethyl ether (1.5 mL) for approx. 90 mins. 1-Bromo-2-methylpropane **48** (6.00 mL, 7.53 g, 55.0 mmol) was added dropwise as solution in diethyl ether (15 mL) and was stirred at reflux for 40 mins upon completion of the addition. The reaction mixture was cooled to 0 °C and benzaldehyde (5.0 mL, 5.20 g, 49.0 mmol) in ether (15 mL) was added slowly, over 15 mins. The reaction was warmed to room temperature and stirred for 90 mins. The mixture was poured onto ice and water and quenched by adding dilute HCl dropwise. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 25 mL). The organic layers were combined and washed with water (3 × 20 mL), brine (75 mL), dried, filtered and concentrated. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure alcohol **46** as a yellow oil (0.47 g, 5%).

ν_{max} (ATR) 3344, 3086, 3029, 2955, 2925 (C–H), 1494, 1453 (C=C) cm^{-1}; δ_H (300 MHz) 0.95 [dd, 6H, *J* = 6.3, 1.4, CH(C**H**$_3$)$_2$], 1.40–1.61 [m, 1H, C**H**(CH$_3$)$_2$], 1.62–1.90 (m, 2H, C**H$_2$**CH), 4.73 (d, 1H, *J* = 8.1, C**H**OH), 4.75 (d, 1H, *J*= 8.1, CHO**H**), 7.20–7.40 (m, 5H, ArH) ppm; δ_C (75 MHz) 22.3 (2 × CH$_3$), 24.8 [CH(**C**H$_3$)$_2$], 48.3 [**C**H(CH$_3$)$_2$], 72.8 (CHOH), 127.5, 128.5, 125.9 (3 × aromatic CH), 145.2 (aromatic C) ppm.

Methyl 3-methyl-2-phenylbutanoate **42**[4]

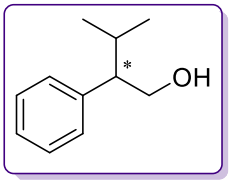


Potassium *tert*-butoxide (8.705 g, 77.7 mmol) was suspended in dry DMF (100 mL) at 0 °C under a nitrogen atmosphere and methyl phenylacetate **43** (9.4 mL, 10 g, 66.6 mmol) was added at once, followed by isopropyl bromide (6.30 mL, 8.25 g, 67.1 mmol) after 2 min. The ice bath was removed and the reaction allowed warm to room temperature (ca. 20 °C) and stirring was continued for 1 h. The reaction was quenched with water (50 mL) and diluted with DCM (40 mL), and stirred overnight. The solution was extracted with DCM (2 × 40 mL). The organic layer was washed with sat. aq. NH_4Cl solution (40 mL) and water (40 mL), dried, filtered and concentrated to give crude **42** as a pale yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether (90/10) as eluent gave pure ester **42** (8.358 g, 68%) as a colorless oil.

δ_H (300 MHz) 0.70 [d, 3H, *J* = 6.6, one of C(4)H$_3$], 1.03 [d, 3H, *J* = 6.6, one of C(4)H$_3$], 2.25–2.45 [m, 1H, C(3)H], 3.15 [d, 1H, *J* = 10.5, C(2)H], 3.65 (s, 3H, OCH$_3$), 7.18–7.41 (m, 5H, ArH) ppm; δ_C (75 MHz) 20.2 [one of C(4)H$_3$], 21.5 [one of C(4)H$_3$], 28.0 [C(2)H], 51.7 (OCH$_3$), 60.0 [C(1)], 127.2, 128.4, 128.5 (3 × aromatic CH), 138.4 (aromatic C), 174.4 (C=O) ppm.

All data is in agreement with previously reported data.[4]

3-Methyl-2-phenylbutan-1-ol **3**[4]



Methyl 3-methyl-2-phenylbutanoate **42** (8.0 g, 41.6 mmol) in diethyl ether (40 mL) was added slowly to a stirring suspension of $LiAlH_4$ (3.30 g, 87.0 mmol) in diethyl ether (80 mL). The reaction was monitored by TLC and after complete consumption of the ester (6 h), the reaction was quenched with ethyl acetate (40 mL). The solution was stirred, water (40 mL) was added and the volatiles removed. The layers were separated, and the aqueous layer was extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried, filtered and concentrated to give the alcohol **3** (6.83 g, 100%) as a colourless oil, which was used without purification.

The spectroscopic characteristics were identical to the ones listed above.

Lipase-catalysed reactions

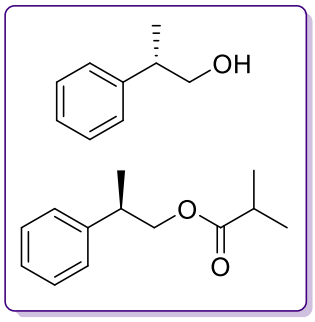
Lipase-catalysed hydrolysis reactions

In a typical experiment 8 mg of powdered lipase, or 18 mg of immobilised lipase, was added to the ester substrates (50 mg) in 0.1 M phosphate buffer, pH 7 (4.5 mL). Cosolvents (HPLC grade, obtained from Sigma Aldrich) were added (0.77 mL, 17% *v*/*v*) as indicated. The small test tubes were sealed and agitated at 750 rpm at 30 °C for 65 h, unless otherwise stated. The aqueous layer was extracted with diethyl ether (3 × 5 mL) and the combined organic extracts were filtered through Celite® and concentrated under reduced pressure. The conversion was determined by ^{1}H NMR; the sample was reconcentrated and dissolved in *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity was determined by chiral HPLC analysis.

Lipase-catalysed transesterification reactions

In a typical experiment 8 mg of powdered lipase, or 18 mg of immobilised lipase, was added to the substrate alcohol (10 mg) in the appropriate vinyl ester (0.5 mL), with crushed 4Å molecular sieves, as indicated. A solvent (HPLC grade, Sigma Aldrich) was added if required and the test tubes were sealed and agitated at 150 rpm for at 30 °C for the times specified in the relevant tables. The reaction mixture was diluted with ether (~5–10 mL), filtered through Celite® and concentrated under reduced pressure. The conversion was determined by ^{1}H NMR; the sample was reconcentrated and dissolved in *n*-hexane/*i*PrOH (90/10, HPLC grade) to a concentration of ~1 mg/mL and enantioselectivity was determined by chiral HPLC analysis as indicated.

Preparative scale lipase-mediated hydrolysis of **24**



Candida antarctica Lipase B (immobilised) (52 mg, 25% *w*/*w*) was added to a solution of 2-phenylpropyl isobutyrate **24** (200 mg, 1.0 mmol) in phosphate buffer (0.1M, pH 7, 10 mL) and *tert*-butanol (1.7 mL, 17% *v*/*v*). The solution was sealed in a conical flask and shaken at 750 rpm for 72 h. The reaction mixture was filtered through Celite® to remove the lipase and the filter cake was washed with diethyl ether (5 × 10 mL). The resulting solution was separated, and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The organic layers were combined and washed with brine (50 mL) and concentrated to give a crude mixture of alcohol *(S)*-**1** and ester *(R)*-**24** (43% conversion, ee_s = 65%, ee_p = 86%). The mixture

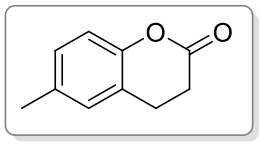
was purified by column chromatography on silica gel using hexane/ethyl acetate as eluent (95/5) to give the pure ester *(R)*-**24** (106 mg, 53%) as a colourless oil $[\alpha]_D^{20}$ −24.2 (c 0.72 in methanol), 70% *ee* and the pure alcohol *(S)*-**1** (45 mg, 34%) as a colourless oil $[\alpha]_D^{20}$ −119.5 (c 0.2 in methanol), 90% *ee*.

^{1}H NMR data for the enantioenriched *(R)*-**24** were identical to that of the racemic sample previously described. Data for *(S)*-**1**:

δ_H (300 MHz) 1.25 (d, 3H, *J* = 7.1, CH_3), 1.73 (broad s, 1H, OH), 2.75–3.05 (sym m, 1H, CH_3), 3.65 (d, 2H, *J* = 7.1, C**H**$_2$OH), 7.10–7.43 (m, 5H, ArH) ppm.

6.3 Synthesis & resolution of 6-methylchroman-2-ol **51**

6-Methylchromanone **75**



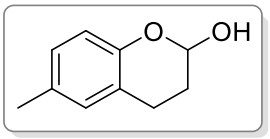
Method 1:[7] Pd/C (10 wt%, 0.072 g) was added to a stirring solution of 6-methylcoumarin **74** (1.030 g, 6.4 mmol) in acetic acid (18.5 mL) and the mixture was stirred under an atmosphere of hydrogen overnight. Reaction completion was shown by disappearance of starting material by TLC (12 h). The crude reaction mixture was filtered through Celite® to remove the catalyst, diluted with water (20 mL) and neutralized by slow addition of solid sodium carbonate. The mixture was extracted with DCM (3 × 25 mL) and the combined organic layers were washed with water (30 mL), brine (30 mL), dried, filtered and concentrated to give the product as a white solid (0.258 g, 25%) which required no further purification (m.p. 78–79 °C, lit. 78–79 °C).[8]

Method 2:[9] Pd/C (10 wt%, 0.556 g, 5 mol%) was added to a stirring solution of 6-methyl coumarin **74** (2.77 g, 16.7 mmol) in ethyl acetate (17.5 mL) and the mixture was stirred under an atmosphere of hydrogen until TLC showed disappearance of starting material (48 h). The suspension was filtered through a bed of Celite® using ethyl acetate (10 mL) and concentrated to give a yellow oil, which solidified on cooling. The solid residue was dissolved in ethyl acetate (10 mL), filtered through Celite®, and concentrated to give a white solid (2.704 g, 97%) which was used without further purification (m.p. 77–78 °C, lit. 78–79 °C).[8]

ν_{max} (ATR) 1740 (C=O), 1500 (C–C), 1209 (C–O), 810 cm^{-1}; δ_H (300 MHz) 2.31 (s, 3H, CH_3), 2.72–2.81 (m, 2H, CH_2), 2.90–3.00 (m, 2H, CH_2), 6.90–7.10 (m, 3H, ArH); δ_C (75 MHz) 20.7, 23.8, 29.4, 116.7, 122.3, 128.5, 128.7,134.0, 150.0 (6 × aromatic C), 168.8 (C=O) ppm.

Data for this compound matches previously reported data.[10]

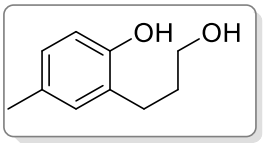
6-Methylchroman-2-ol **51**[11]



Method 1: Diisobutylaluminium hydride (1 M in toluene, 5.8 mL, 5.8 mmol) was added dropwise to a solution of 6-methylchromanone **75** (0.84 g, 5.2 mmol) in distilled toluene (18 mL) at −78 °C under an atmosphere of nitrogen. The mixture was stirred at −78 °C for 3 h. TLC showed that the starting material was still present and an additional portion of DIBAL (3 mL, 3.0 mmol) was added, and the reaction mixture stirred for a further 2 h. The reaction was quenched with water (7 mL) and allowed to warm to room temperature. The mixture was filtered through Celite®, and the filter cake washed with diethyl ether (30 mL) and the filtrate layers were separated. The aqueous layer was extracted with diethyl ether (20 mL) and the organic layers were combined. The organic layer was washed with water (50 mL), brine (2 × 50 mL), dried, filtered, and concentrated to give the product as a yellow oil (0.427 g, 50%). A portion of the product (0.297 g) was purified by column chromatography using hexane/ethyl acetate as an eluent (1/1) giving the pure product **51** (0.213 g) as a yellow oil. The other portion of the product was acylated directly without purification.

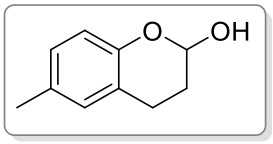
ν_{max} (ATR) 3401 (OH), 2939 (C–H), 1498, 1206 (C–O), 950, 811 cm^{-1}; δ_H (300 MHz) 1.85–2.11 (m, 2H, CH_2), 2.25 (s, 3H, CH_3), 2.66 (dt, *J* = 16.4, 5.3, 1H, CH_2), 2.82–3.04 (m, 1H, CH_2), 3.25 (br s, 1H, OH), 5.58 (br s, 1H, C**H**OH), 6.71 (d, *J* = 8.1, 1H, ArH), 6.78–7.00 (m, 2H, ArH) ppm; δ_C (75 MHz) 20.3, 20.5, 27.1, 92.1, 116.6, 121.7, 128.0, 129.6, 130.1, 149.7 ppm; HRMS (ES^+): $[M-H_2O]^+$ 147.0808 (calculated: 147.0810).

Data for this compound matches previously reported data.[10] The signal at 5.58 ppm sometimes appears as an unresolved quartet (J = 3.0)#.



A minor product (0.109g) was also recovered from the column, R_f = 0.4. It was identified as the diol **76**, corresponding to the reduction of the aldehyde, which exists in equilibrium with the lactol product **51** above. It is believed to have been a result of the extra DIBAL added, and the ^1H NMR data matches previously reported data for this compound.[12]

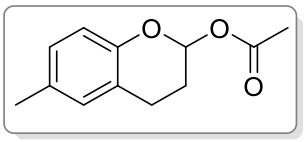
δ_H (300 MHz) 1.80–1.92 (m, 2H, C**H**$_2$$CH_2$OH), 2.25 (s, 3H, Ar$CH_3$), 2.74 (t, 2H, *J* = 6.9, ArCH_2), 3.63 (t, 2H, *J* = 5.8, C**H**$_2$OH), 6.64–6.80 (m, 1.7 H, 1 × ArH & ArOH), 6.84–6.97 (m, 2H, ArH) ppm; δ_C (75.5 MHz), 20.5, 25.1, 32.3, 60.8, 115.9, 129.9, 128.0, 126.9, 131.1, 152.2 ppm.



Method 2: Diisobutylaluminium hydride (1 M in toluene, 11.2 mL, 11.2 mmol) was added dropwise to a stirring solution of 6-methylchromanone **75** (1.56 g, 9.6 mmol) and powdered 3Å molecular sieves in distilled toluene (30 mL) at −78 °C under an atmosphere of nitrogen. The mixture was stirred for 6 h. The reaction was quenched with water (5 mL) and allowed to warm to room temperature. The solution was filtered through Celite®, the filter cake washed with diethyl ether (50 mL) and the filtrate layers were separated. The aqueous layer was extracted with diethyl ether (50 mL). The combined organic layer was washed with water (2 × 50 mL), brine (100 mL), dried, filtered, and concentrated to give the product **51** as a colourless oil (0.93 g, 59%).

The spectroscopic data were identical to those reported above.

6-Methylchroman-2-yl acetate **62**



Acetic anhydride (0.6 mL, 0.648 g, 6.4 mmol, 6.7 eq.), DMAP (1.1 mg, 0.01 mmol) and pyridine (0.34 mL, 0.334 g, 4.2 mmol, 4.5 eq.) were added to a solution of lactol **51** (154.9 mg, 0.944 mmol) in DCM (3.5 mL). The resulting solution was stirred for 22 h. Sat. aq. $NaHCO_3$ (10 mL) was added to the solution and stirred vigorously until effervescence ceased (approx. 30 mins). The layers were separated, and the aqueous layer was extracted with DCM (10 mL). The combined organic layer was washed with sat. aq. copper sulfate solution (20 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), brine (20 mL), dried, filtered and concentrated to give an orange/yellow oil (0.291 g). Excess pyridine was removed by azetropic distillation with heptane (3 × 10 mL) to give the crude product **62** (0.161 g, 83%) as a dark yellow oil. The product was purified by chromatography on silica gel using hexane/ethyl acetate (5/1) as an eluent, which gave the pure product **62** as a pale yellow oil (0.085 g, 44%).

ν_{max} (ATR) 2933 (C–H), 1748, (C=O), 1498, 1199, 1179 cm^{-1}; δ_H (300 MHz) 1.90–2.18 (m, 5H, CH_2 & CH_3), 2.26 (s, 3H, CH_3), 2.57–2.74 (m, 1H, CH_2), 2.86–3.03 (m, 1H, CH_2), 6.50 (t, 1H, *J* = 2.6, 1H, C**H**OCH_3), 6.76 (d, 1H, *J* = 8.2, ArH), 6.84–6.98 (m, 2H, ArH) ppm; δ_C (75.5 MHz) 19.7, 20.5, 21.2, 25.2, 90.3, 116.8, 121.3, 128.2, 129.6, 130.7, 149.3, 169.9 ppm; HRMS (ES^+): $[M-OAc]^+$ 147.0816 (calculated: 147.0816); enantiomers separated using Phenomenex Cellulose 4 [conditions: *n*-hexane/*i*PrOH = 95/5, flow rate = 1 mL min^{-1}], (−)-**62** R_t = 6.0, (+)-**62**, R_t = 6.5 min.

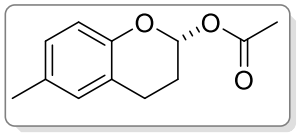
This compound is novel and has been fully characterized in this investigation.

Enzymatic resolutions

General procedure for enzymatic transesterification screens

The substrate **51** (10 mg) was added to a small test tube. The acyl source (0.5 mL) and solvent (2 mL, if applicable) were then added, along with a spatula tip of enzyme (~8mg powdered, or 18 mg immobilised). The reaction was sealed and incubated in a mini-shaker at 30 °C, at 150 rpm. When the stipulated time period had elapsed, the solution was passed through a Pasteur pipette containing a layer each of Celite® and $MgSO_4$, using diethyl ether as eluent. The solvent was removed under reduced pressure and the resulting crude mixture was analysed by ^1H NMR spectroscopy for conversion data and chiral HPLC for enantioselectivity.

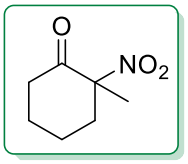
Preparative scale resolution of (+)-6-methylchroman-2-yl acetate (+)-62



6-Methylchromanol **51** (50 mg) was added to a small test tube. Vinyl acetate (50 eq.) and hexane (4 mL) were added to a test tube with lipase from *Thermomyces lanuginosus* (40 mg) as the biocatalyst. The test tube was sealed, and the reaction was incubated at 30 °C for 4 days. The crude reaction mixture was passed through a Pasteur pipette, as above, and the solvent was removed in under reduced pressure. ^1H NMR analysis of the crude mixture indicated a 65% conversion. The crude mixture was purified using column chromatography with hexane/ethyl acetate (5/1) as eluent to give pure **62** in 32% yield and 94% *ee*. $[\alpha]_D^{20}$= +26.7 (c 0.2, $CHCl_3$).

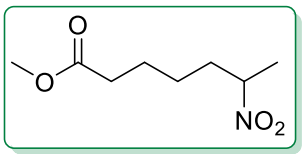
6.4 Synthesis & resolution of 2-methyl-2-nitrocyclohexanol **85a** & **85b**

2-Methyl-2-nitrocyclohexanone **89**[13]



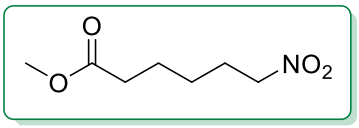
2-Nitrocyclohexanone **88** (2.09 g, 14.6 mmol) in DCM (19 mL) was added in one portion to a vigorously stirring solution of tetrabutylammonium hydroxide (11.33 g, of a 40% aq. solution, 17.5 mmol) in water (17 mL) under nitrogen. The reaction mixture was stirred for 10 min, and then methyl iodide (4.4 mL, 10.03 g, 70.7 mmol) was added in one portion. The reaction mixture was stirred vigorously for 48 h at room temperature. The reaction mixture was then transferred to a separating funnel and layers separated. The organic layer was washed with water (20 mL). The aqueous phase was extracted with DCM (2 × 30 mL) and the combined organic fractions were dried, filtered and concentrated under reduced pressure at room temperature. Diethyl ether (50 mL) was added to precipitate the tetrabutylammonium iodide salt, the supernatant was filtered and concentrated under reduced pressure at room temperature to give a crude mixture (2.08 g) of 2-methyl-2-nitrocyclohexanone **89** and ring cleavage products, methyl 6-nitrohexanoate **90** and methyl 6-nitroheptanoate **91** (77:15:8 respectively) as a viscous brown oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure α-nitro ketone **89** (0.754 g, 33%) as a colourless oil.

ν_{max} (ATR) 2946, 2872, 1726 (C=O), 1543 (NO_2), 1386 (NO_2) cm^{-1}; δ_H (300 MHz) 1.67 [s, 3H, C(2)CH_3], 1.69–1.85 (m, 4H, ring protons), 1.98–2.10 (m, 1H, ring protons), 2.55–2.64 (m, 2H, ring protons), 2.86–2.90 (1H, m, ring protons); δ_C (75.5 MHz) 21.5, 22.4, 26.6, 38.4, 39.2, 93.9, 200.7 ppm.



The second fraction isolated from the column was methyl 6-nitroheptanoate **91** as a pale yellow oil (269 mg).

ν_{max} (ATR) 2951, 1733 (C=O), 1546 (NO_2), 1390 (NO_2) cm^{-1}; δ_H (300 MHz) 1.30–1.45 (m, 1H, CH_2), 1.53 (d, 3H, J = 6.6, CH_3), 1.57–1.83 [m, 3H, C(5)H & C(3)H_2], 1.91–2.13 [m, 1H, C(5)H], 2.32 [t, 2H, J = 7.4, C(2)H_2], 3.67 (s, 3H, OCH_3), 4.47–4.67 [sym m, 1H, C(6)H] ppm; δ_C (75.5 MHz) 19.2, 24.2, 25.2, 33.6, 34.7, 51.5, 83.3, 173.6 ppm.

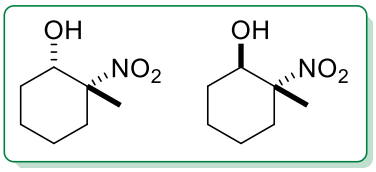


The third fraction isolated from the column was methyl 6-nitrohexanoate **90** as a yellow oil (197 mg).

ν_{max} (ATR) 2951, 1733 (C=O), 1548 (NO_2), 1382 (NO_2) cm^{-1}; δ_H (300 MHz) 1.33–2.19 (m, 5H, alkyl CH), 2.34 [t, 2H, *J* = 7.4, C(5)H_2], 3.68 (s, 3H, OCH_3), 4.39 [t, 2H, *J* = 7.0, C(2)H_2] ppm; δ_C (75.5 MHz) 24.2, 25.7, 27.0, 33.5, 51.6, 75.4, 173.6 ppm.

Data is consistent with literature values.[14]

2-Methyl-2-nitrocyclohexanol **82a** & **82b**[15]



A solution of 2-methyl-2-nitrocyclohexanone **89** (1.01 g, 6.4 mmol) in ethanol (11 mL) was added dropwise over 10 min to a stirred suspension of $NaBH_4$ (0.267 g, 7.1 mmol) in ethanol (11 mL) at 0 °C under nitrogen and stirring was continued for 1.5 h at 0 °C. The ice bath was then removed and aq. HCl (10%) was added to adjust to pH 1. The solution was concentrated under reduced pressure and the resulting residue was partitioned between water (20 mL) and DCM (20 mL). The aqueous phase was extracted with DCM (3 × 20 mL) and the combined organic extracts were washed with brine (30 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (1.00 g) of nitroalcohols *cis*-**82a** and *trans*-**82b** (3:1 respectively) as a yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether 60/40 as eluent gave *cis*-2-methyl-2-nitrocyclohexanol **82a** (0.450 g, 44%) as a viscous light yellow oil.

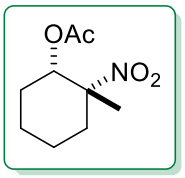
ν_{max} (ATR) 3429 (OH), 2944 (C–H), 1533 (NO_2), 1357 (NO_2) cm^{-1}; δ_H (300 MHz) 1.36–1.57 [m, 3H, one of C(5)H_2 & C(4)H_2], 1.63 [s, 3H, C(2)CH_3], 1.66–1.94 [m, 4H, one of C(5)H_2, one of C(3)H_2 and C(6)H_2], 2.46 [dt, 1H, *J* = 13.9, 6.0, one of C(3)H_2], 2.79 [d, 1H, *J* = 8.3, OH], 3.91 [td, 1H, *J* = 8.0, 3.6, C(1)H] ppm; δ_C (75.5 MHz) 21.6 [CH_2, C(5)], 21.9[CH_2, C(4)], 24.2[CH_3, C(2)**C**H_3], 30.6 [CH_2, C(6)], 33.0 [CH_2, C(3)], 73.1[CH, C(1)], 91.4 [qC, C(2)] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL min^{-1}], R_t = 40.1 min, 44.1 min.

A fraction containing both *cis*-**82a** and *trans*-**82b** (70:30) was also isolated, this was used for enzymatic screens, as it is approximately equal to the thermodynamic ratio which is achieved when subjecting equimolar mixture of *cis*-**82a** and *trans*-**82b** to the interconversion conditions.

trans-2-Methyl-2-nitrocyclohexanol **82b** (81 mg, 8%) was isolated as a colourless oil.

ν_{max} (ATR) 3429 (OH), 2944 C–H), 1537 (NO_2), 1357 (NO_2) cm^{-1}; δ_H (300 MHz) 1.29–1.53 [m, 3H, one of C(4)H_2, one of C(5)H_2 and one of C(6)H_2], 1.61 [s, 3H, C(2)CH_3], 1.66–2.05 [m, 4H, one of C(4)H_2 & one of C(5)H_2 & one of C(3)H_2 & one of C(6)H_2], 2.05–2.23 [m, 1H, one of C(3)H_2], 2.77 [br s, 1H, OH], 4.21–4.34 [m, 1H, C(1)H] ppm; δ_C (75.5 MHz) 16.3 [CH_3, br, C(2)*C*H_3], 22.1 [CH_2, C(4)H_2], 23.5 [CH_2, C(5)H_2], 30.6 [CH_2, C(6)H_2], 35.9 [CH_2, br, C(3)H_2], 72.4 [CH, C(1)H], 93.0 [qC, C(2)] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL min^{-1}], R_t = 47.1 min, 53.5 min.

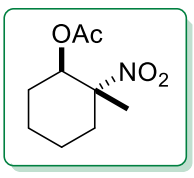
cis-2-Methyl-2-nitrocyclohexyl acetate **87a**



DMAP (1 mg) was added to a stirring solution of *cis*-2-methyl-2-nitrocyclohexanol **82a** (41 mg, 0.3 mmol), acetic anhydride (0.15 mL, 1.6 mmol) and pyridine (0.1 mL, 1.2 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 21 h. Saturated aq. $NaHCO_3$ (3 mL) was added and the mixture stirred for 30 min. The solution was transferred to a separating funnel and washed with sat. aq. $CuSO_4$ (6 mL), water (6 mL), sat. aq. $NaHCO_3$ (6 mL) and brine (6 mL). The organic extract was dried, filtered and concentrated under reduced pressure to give crude acetate **87a** (27 mg, 51%) as a colourless oil which was sufficiently pure to use without further purification.

δ_H (300 MHz) 1.33–1.56 [m, 3H, C(5)H_2, and one of C(4)H_2], 1.65 [s, 3H, C(2)CH_3], 1.59–1.90 [m, 2H, one of C(4)H_2 and one of C(6)H_2], 2.03 [s, 3H, $COCH_3$], 1.91–2.22 [m, 3H, one of C(6)H_2 and C(3)H_2], 5.53 [dd, 1H, *J* = 9.9, 4.5, C(1)H] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL min^{-1}], R_t = 23.5 min, 26.8 min.

trans-2-Methyl-2-nitrocyclohexyl acetate **87b**



This was prepared following the procedure for **82a**, from DMAP (1 mg), *trans*-2-methyl-2-nitrocyclohexanol **82b** (43 mg, 0.3 mmol), acetic anhydride (0.15 mL, 1.6 mmol) and pyridine (0.1 mL, 1.2 mmol) in DCM (2 mL) to give the crude acetate **87b** (26 mg, 48%) as a light yellow oil which was sufficiently pure to use without further purification.

δ_H (300 MHz) 1.34–1.57 [m, 3H, one of C(4)H_2, one of C(5)H_2 and one of C(6)H_2], 1.61 (s, 3H, CH_3), 1.62–1.93 [m, 2H, one of C(5)H_2 and one of C(4)H_2], 2.02 (s, 3H, OCH_3), 1.95–2.14 [m, 3H, C(3)H_2 and one of C(6)H_2], 5.29–5.40 [m, 1H, C(1)H]; enantiomers separated using

Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL min^{-1}], R_t = 17.8 min, 19.1 min.

Lipase-mediated kinetic resolution

General procedure for the development of one-pot procedures: lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol 82a or 82b with vinyl acetate as acetylating agent and dynamic interconversion process (analytical scale)

In a typical experiment, the following were added as appropriate to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** (typically 15 mg) in the appropriate solvent (10 mg/mL): DBU (immob) (0.5 eq.) (18 mg) or vinyl acetate, and CAL-B (immob) (approx. 15 mg). The small test tube was sealed and agitated as appropriate for the specified length of time (generally 12 h). The solutions were filtered through Celite® and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

General procedure for the development of two-pot procedures: dynamic interconversion process and lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol cis-82a and trans-82b with vinyl acetate 65 as acetylating agent (analytical scale)

In a typical experiment, DBU (immob) (0.5 eq., ~90 mg) was added to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** in toluene (5 mL, 10 mg/mL) and the test tube was sealed and shaken at 500 rpm for the required amount of time at the specified temperature. The reaction solution was filtered through Celite®, concentrated under reduced pressure and redissolved in the appropriate amount of solvent, if applicable. CAL-B (immob) and vinyl acetate were added and the solution shaken at the appropriate speed and temperature for the required amount of time in a sealed test tube. The solution was filtered through a plug of Celite® and concentrated under reduced pressure if applicable. The samples were taken before each filtration and filtered through Celite®, concentrated under reduced pressure. The samples were analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

General procedure for the development of one-pot procedures: dynamic interconversion process and lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol cis-82a and trans-82b with vinyl acetate as acetylating agent (analytical scale)–cycling procedure

In a typical experiment, immobilised DBU (0.5 eq., ~90 mg) was added to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** in toluene (5 mL, 10 mg/mL) and the test tube was sealed and shaken at 50 °C, 500 rpm for the required amount of time. CAL-B (immob) (approx. 100 %

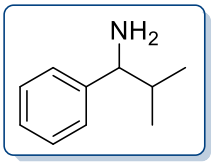
w/w) and vinyl acetate **65** (3.0 eq.) was added and the test tube was sealed, shaken at 400 rpm for 12 h at 30 °C. At the end of the cycle the solution was filtered through a plug of Celite® if appropriate and the filtrate was recycled through the process again. Before the addition of each reagent an aliquot was taken, filtered through Celite®, concentrated under reduced pressure. The samples were analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

Diastereoselective lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol 82

CAL-B (immob) (88.6 mg) was added to an equimolar mixture of *cis*- and *trans*-2-methyl-2-nitrocyclohexanol (±)-**82a** and (±)-**82b** (105.4 mg, 0.7 mmol) dissolved in vinyl acetate (5 mL). The reaction mixture was shaken at 750 rpm at room temperature. Reaction monitoring was conducted as follows: an aliquot (0.5 mL) of reaction mixture was isolated and filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure and the sample was analysed by ^{1}H NMR spectroscopy. The final extraction following ^{1}H NMR spectroscopy was dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

6.5 Synthesis of transaminase substrates

2-Methyl-1-phenylpropan-1-amine **114**

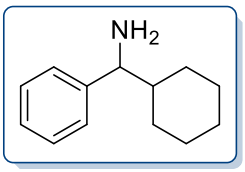


A mixture of 2-methylpropiophenone (0.740 g, 5.0 mmol), titanium(IV) ethoxide (2.1 mL, 2.28 g, 10.0 mmol) and ammonia (2 M in methanol, 12.5 mL, 25.0 mmol) was stirred under nitrogen, at ambient temperature for 15 h. Sodium borohydride (0.288 g, 7.6 mmol) was added and the resulting mixture was stirred at room temperature for an additional 3 h. The reaction was quenched by pouring onto ammonium hydroxide (2 M, 12.5 mL), the resulting inorganic precipitate was removed by filtration, and washed with ethyl acetate (2 × 15 mL). The aqueous solution was extracted with ethyl acetate (2 × 15 mL). The combined organic solution was extracted with aq. HCl (10%, 15 mL). The acidic aqueous extracts were washed with ethyl acetate (25 mL), then treated with aq. NaOH (2 M) to pH 10–12 and extracted with ethyl acetate (3 × 25mL). The combined organic extracts were washed with brine (25 mL), dried (Na_2SO_4), filtered and concentrated to give the primary amine **114** as a colourless oil (0.368 g, 49%), which required no further purification.

ν_{max} (ATR) 2957, 2870, 1465, 1451, 701 cm^{-1}; δ_H (300 MHz) 0.77 (d, 3H, *J* = 6.7, CH_3), 0.98 (d, *J* = 6.7, 3H, CH_3),1.50 (br s, 2H, NH_2), 1.75–1.96 (sym m, 1H, C**H**$(CH_3)_2$), 3.60 (d, 1H, *J* = 7.3, C**H**NH_2), 7.06–7.53 (m, 5H, ArH) ppm; δ_C (75.5 MHz) 18.9 (CH_3), 19.8, 35.5, 62.5, 126.8, 127.0, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm.

All data is in agreement with previously reported data.[16]

Cyclohexyl(phenyl)methanamine **115**



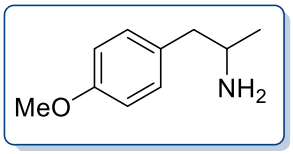
Prepared following the procedure for **114** using cyclohexyl(phenyl)ketone (1.878 g, 10.0 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.567 g, 15.0 mmol) to give the product amine **115** as a white solid (0.437 g, 23%), which required no further purification, (m.p. 99–102 °C).

ν_{max} (ATR) 3421, 2922, 2849, 1552, 1453, 1357, 704, 528 cm^{-1}; δ_H (300 MHz) 0.73–1.71 (m, 12H, ring CH & NH_2), 1.71–1.83 (m, 1H, ring CH), 1.86–2.04 (m, 1H, ring CH), 3.60 (d, 1H, *J* = 7.5, C**H**NH_2), 7.10–7.48 (m, 5H, ArH) ppm; δ_C (75.5 MHz) 26.2, 26.4, 29.5, 30.1 (4 × CH_2), 45.2, 61.7 (2 × CH), 126.8, 127.1, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm.

^{13}C signal at 145.5 ppm is very weak and was confirmed by HMBC correlation experiments. Carbon assignments were aided by DEPT experiments.

All data is in agreement with previously reported data.[17] Melting point was not previously reported.

1-(4-Methoxyphenyl)propan-2-amine **121**[18]



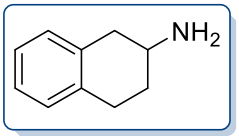
Prepared following the procedure for **114** using 4′-methoxyphenylacetone **148** (1.640 g, 10.0 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.575 g, 15.2 mmol) to give the product amine **121** as a pale yellow oil (0.559 g, 34%), which required no further purification.

ν_{max} (ATR) 2957, 2835, 1611, 1510, 1242, 1176, 1033, 802 cm^{-1}; δ_H (300 MHz) 1.10 (d, 3H, *J* = 6.3, CH_3), 2.46 (dd, 1H, *J* = 13.4, 8.0, one of CH_2), 2.65 (dd, 1H, *J* = 13.4, 5.3, one of CH_2), 3.04–3.21 (m, 1H, C**H**NH_2), 3.79 (s, 3H, OCH_3), 6.70–6.94 (m, 2H, ArH), 7.02–7.33 (m, 2H, ArH) ppm; δ_C (75.5 MHz) 23.5, 45.8, 48.6, 55.3, 113.8, 130.2, 131.8, 158.1 ppm; enantiomers separated

using Amylose 1 column [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.4 mL min^{-1}], R_t = 16.0, R_t = 16.8 min, R_t (ketone) = 14.4 min.

All data is in agreement with previously reported data.[19]

1,2,3,4-Tetrahydronaphthalen-2-amine **130**

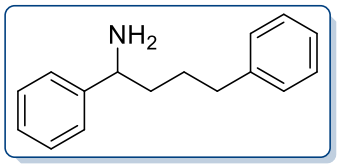


Prepared following the procedure for **114** using 2-tetralone **131** (1.472 g, 10.1 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.584 g, 15.4 mmol) to give the product amine **130** as a dark green oil (0.713 g, 48%), which was used without further purification.

ν_{max} (ATR) 3354, 2918, 2841, 741 cm^{-1}; δ_H (300 MHz) 1.51–1.74 [m, 3H, one of C(3)H_2 and NH_2 (1.66, br s)], 1.91–2.10 [m, 1H, one of C(3)H_2], 2.56 [dd, 1H, *J* = 16.1, 9.4, one of C(1)H_2], 2.77–2.94 [m, 2H, C(4)H_2], 3.00 [ddd, 1H, *J* = 16.2, 5.0, 1.3, one of C(1)H_2], 3.18 (tdd, 1H, *J* = 9.5, 5.0, 3.1, C**H**NH_2), 6.87–7.22 (m, 4H, ArH) ppm; δ_C (75.5 MHz) 28.1, 33.0, 39.5 (3 × CH_2), 47.3 (CH), 125.7, 125.8, 128.7, 129.3 (4 × aromatic CH), 135.3, 135.9 (2 × aromatic C) ppm.

All data is in agreement with previously reported data.[20] Proton and carbon assignments were aided by 2D NMR and DEPT experiments.

1,4-Diphenylbutan-1-amine **116**

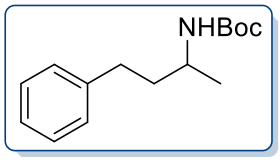


Prepared following the procedure for **114** using 1,4-diphenylbutanone (0.451 g, 2.0 mmol), titanium(IV) ethoxide (0.84 mL, 0.91 g, 4.0 mmol), ammonia (2 M in methanol, 5 mL, 10.0 mmol) and sodium borohydride (0.122 g, 3.2 mmol) to give the product amine **116** as a colourless oil (0.074 g, 16%), which required no further purification.

ν_{max} (ATR): 2934, 1265, 732, 699 cm^{-1}; δ_H (300 MHz) 1.38–1.98 [m, 6H, 2 × CH_2 & NH_2 (1.69, s)], 2.48–2.71 (m, 2H, CH_2), 3.77–3.97 (m, 1H, C**H**NH_2), 6.91–7.51 (m, 10H, ArH) ppm; δ_C (75.5 MHz), 28.4, 35.8, 39.1 (3 × CH_2), 56.3 (CH), 125.7, 126.4, 127.0, 128.3, 128.4, 128.5 (6 × aromatic CH), 142.3, 146.3 (2 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 226.1589 (calculated: 226.1596).

This compound has been fully characterized in this investigation.

tert-Butyl-(4-phenylbutan-2-yl)carbamate **158**[21]

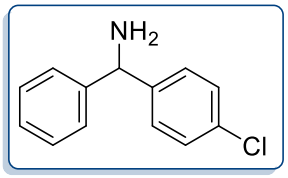


A solution of 4-phenyl-2-aminobutane **120** (16.18 µL, 0.1 mmol) in DCM (2 mL) was added to a solution of di-*tert*-butyl-dicarbonate (21.83 mg, 0.1 mmol, 1.0 eq.) in DCM (2 mL). The reaction solution was stirred for 3 h at room temperature. The solvent was evaporated to give the crude N-Boc-amine **158** as a colourless oil which was used without further purification for chiral HPLC method development.

δ_H (300 MHz) 1.16 [d, 3H, *J* = 6.2, C(1)H_3], 1.45 [s, 9H, C(CH_3)$_3$], 1.62–1.82 (m, 2H, CH_2), 2.55–2.80 (m, 2H, CH_2), 3.72 (br s, 1H, C**H**NH_2), 4.37 (br s, 1H, NH_2), 7.07–7.37 (m, 5H, ArH) ppm.

All data is in agreement with previous reports.[22]

(4′-Chlorophenyl)(phenyl)methanamine **118**

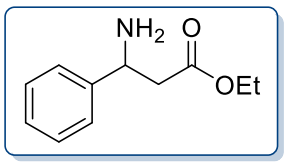


Prepared by Gavin following the procedure for **114** using 4′-chlorobenzophenone (1.08 g, 5.0 mmol), titanium(IV) ethoxide (2.1 mL, 2.28 g, 10.0 mmol), ammonia (2 M in methanol, 12.5 mL, 25.0 mmol) and sodium borohydride (0.3 g, 8.0 mmol) to give the product **118** as a colourless oil.

δ_H (300 MHz) 1.73 (v br s, 2H, NH_2), 5.12 (br s, 1H, C**H**NH_2), 7.49–7.09 (m, 9H, ArH) ppm;

All data is in agreement with previously reported data.[23]

Ethyl 3-amino-3-phenylpropanoate **117**



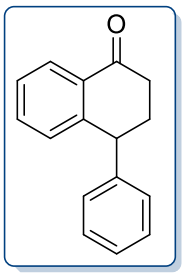
Sulfuric acid (conc., 2.4 mL, 4.42 g, 45.0 mmol) was added to a solution of 3-amino-3-phenylpropanoic acid **133** (2.48 g, 15.0 mmol) in absolute ethanol (50 mL) and heated under reflux for 24 h. Excess ethanol was evaporated under reduced pressure and the crude product was dissolved in DCM (50 mL) and washed with water (2 × 50 mL), sat. aq. $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried, filtered and concentrated to give the ester **117** as a colourless oil (0.999 g, 34%) which was used without further purification.

ν_{max} (ATR) 3381, 1726 (C=O), 1178, 1031, 699, 539 cm^{-1}; δ_H (300 MHz) 1.23 (t, 3H, *J* = 7.1, CH_2C**H**$_3$), 1.77 (br s, 2H, NH_2), 2.66 (d, 2H, *J* = 6.9, $COCH_2$), 4.14 (q, 2H, *J* = 7.1, OC**H**$_2$$CH_3$), 4.42

(dd appears as a t, 1H, *J* = 6.8, C**H**NH_2), 7.14–7.48 (m, 5H, ArH) ppm; δ_C (75.5 MHz) 14.2, 44.2, 52.6, 60.6, 126.2, 127.4, 128.6, 144.7 (4 × aromatic C), 172.1 (C=O) ppm.

All data is in agreement with previously reported data.[24]

4-Phenyl-3,4-dihydronaphthalen-1(*2H*)-one **134**[25]

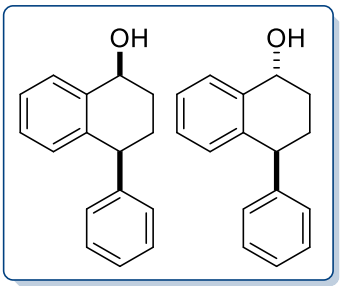


Triflic acid (50 g, 333.0 mmol) was added slowly to a stirring solution of *trans*-styrylacetic acid **142** (11.35 g, 70.0 mmol) and benzene (6.3 mL, 5.47 g, 70.0 mmol) in DCM (50 mL), at 0 °C. The reaction solution was allowed to warm to room temperature and stirred for 24 h. The reaction solution was quenched by pouring onto a mixture of water and ice (50 mL) and the layers separated. The solution was extracted with DCM (3 × 50 mL), and the combined extracts were dried, filtered, and concentrated to give the crude product (14.808 g) which was purified by column chromatography using hexane/ethyl acetate (90/10) as eluent to give the pure product **134** (5.53 g, 36%) as a white solid (m.p.: 69–72 °C, lit.: 70–72 °C).[26]

ν_{max} (ATR) 2917 (C-H), 1682 (C=O), 762, 702 cm^{-1}; δ_H (300 MHz) 2.20–2.38 [m, 1H, one of C(3)H_2], 2.38–2.54 [m, 1H, one of C(3)H_2], 2.54–2.81 [sym m, 2H, C(2)H_2], 4.30 [dd, 1H, *J* = 8.0, 4.6, 1H, C(4)H], 6.98 (d, 1H, *J* = 7.7, 1H, ArH), 7.06–7.16 (m, 2H, ArH), 7.19–7.49 (m, 5H, ArH), 8.12 (dd, 1H, *J* = 7.6, 1.1, ArH) ppm; δ_C (75.5 MHz) 31.9 [C(3)H_2], 36.8 [C(2)H_2], 45.3 [C(4)H], 126.8, 127.06, 127.13, 128.6, 128.7, 129.6 (6 × aromatic CH), 132.9 (aromatic C), 133.6 (aromatic CH), 143.7, 146.3 (2 × aromatic C), 198.1 (C=O) ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL min^{-1}], R_t = 18.5 min; 31.9 min.

All data is in agreement with previously reported data. [26,27]

cis-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-ol *cis*-**137a** & *trans*-4-phenyl-1,2,3,4-tetrahydronaphthalen-1-ol *trans*-**137b**[28]



Sodium borohydride (0.697 g, 18.4 mmol) was added to a stirring solution of ketone **134** (3.823 g, 16.7 mmol) in methanol (50 mL) at 0 °C. The solution was allowed to slowly warm to room temperature and was stirred overnight. The reaction mixture was acidified to pH = 5 using aq. HCl (10%), and the volatiles were removed by rotary evaporation. The

solution was extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with brine (50 mL), dried, filtered and concentrated to give the crude material as a viscous yellow oil (4.533 g) containing a mixture of *cis*-**137a** and *trans*-**137b** diastereomers (46:54). The product *trans*-**137b** (1.46 g, 39%) was obtained as a white solid (m.p.: 121–122 °C, lit.: 122–123 °C)[29] by recrystallisation of the crude mixture from diethyl ether and hexane. The *cis*-enriched material (24:76) which remained in the mother liquor was subjected to column chromatography using diethyl ether/hexane (15/85) as eluent to give the pure product *cis*-**137a** as a colourless oil (0.754 g, 20%).

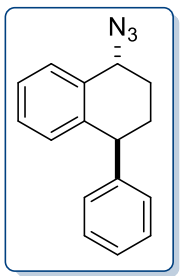
cis-**137a**: ν_{max} (ATR) 3307, 760, 731, 699 cm^{-1}; δ_H (300 MHz) 1.81 (br s, 1H, OH), 1.89–2.25 [m, 4H, C(2)H_2 & C(3)H_2], 4.02 [dd, 1H, *J* = 8.3, 5.6, C(4)H], 4.87 [br s, 1H, C(1)H], 6.85 (d, 1H, *J* = 7.7, ArH), 7.07–7.38 (m, 7H, ArH), 7.46 (m, 1H, ArH) ppm; δ_C (75.5 MHz) 28.3 (CH_2), 30.3 (CH_2), 45.8 [C(4)H], 68.2 [C(1)H], 126.3, 126.6, 127.9, 128.4, 128.8, 128.9, 130.0 (7 × aromatic CH), 139.0, 139.8, 146.6 (3 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 225.1279 (calculated: 225.1274).

^{1}H NMR data matches previously reported data.[30] ^{13}C NMR data was not previously reported and assignments were aided by DEPT and 2D experiments.

trans-**137b**: ν_{max} (ATR) 3230 (OH), 745, 696 cm^{-1}; δ_H (300 MHz) 1.66–1.96 [m, 3H, one of C(2)H_2 and one of C(3)H_2 and OH (1.73, d, *J* = 6.0)], 2.08–2.25 [m, 1H, one of C(2)H_2], 2.27–2.43 [m, 1H, one of C(3)H_2], 4.13–4.22 [m, 1H, C(4)H], 4.85–4.96 [m, 1H, C(1)H], 6.88 (d, 1H, *J* = 7.9, ArH), 6.99–7.06 (m, 2H, ArH), 7.09–7.37 (m, 5H, ArH), 7.55 (d, 1H, *J* = 7.6, ArH) ppm; δ_C (75.5 MHz) 29.2 [C(3)H_2], 30.3 [C(2)H_2], 45.3 [C(4)H], 68.5 [C(1)H], 126.1, 126.7, 127.7, 127.9, 128.3, 128.7, 130.2 (7 × aromatic CH), 139.1, 139.7, 146.5 (3 × aromatic C) ppm; elemental analysis (Found C 85.44; H 7.17. $C_{16}H_{16}O$ requires C 85.68, H 7.19%).

^{1}H NMR data matches previously reported data.[30] ^{13}C NMR data was not previously reported and assignments were aided by DEPT spectra and 2D experiments.

trans 1-Azido-4-phenyl-1,2,3,4-tetrahydronaphthalene *cis*-**140b**[31]



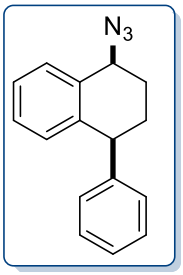
Diphenylphosphoryl azide (1.05 mL, 1.34 g, 4.9 mmol, 1.2 eq.) was added to a solution of *cis*-**137a** (0.914 g, 4.1 mmol) in dry toluene (10 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.73 mL, 0.74 g, 4.9 mmol) was added dropwise to the stirring solution. The mixture was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in

DCM/hexane (1/2) and filtered through a short silica gel pad (approx. 100 mL solvent used), and the solvent was removed in vacuo to give the crude product as a yellow oil (1.065 g). The product was purified by column chromatography using hexane/ethyl acetate (95/5) as eluent to give the pure product *trans*-**140b** (902 mg, 90%) as a colourless oil.

ν_{max} (ATR) 2939, 2091, 1491, 1239, 749, 700 cm^{-1}; δ_H (300 MHz) 1.80–1.96 [2H, m, one of C(2)H_2 and one of C(3)H_2], 2.05–2.23 [1H, m, one of C(2)H_2], 2.30–2.49 [1H, m, one of C(3)H_2], 4.22 [t, 1H, *J* = 5.8, C(4)H], 4.67 [t, 1H, *J* = 5.4, C(1)HN_3], 6.94 (d, 1H, *J* = 7.8, ArH), 6.96–7.03 (m, 2H, ArH), 7.15–7.32 (m, 5H, ArH), 7.38–7.45 (m, 1H, ArH) ppm; δ_C (75.5 MHz) 26.1 (CH_2), 28.9 (CH_2), 44.5 [C(4)H], 59.7 [C(1)HN_3], 126.2, 126.7, 128.32, 128.34, 128.63, 128.64, 130.7 (7 × aromatic CH), 134.6, 139.3, 146.4 (3 × aromatic C) ppm; HRMS (ES^+): $[M-N_3]^+$ 207.1168 (calculated: 207.1170).

This compound is novel and has been fully characterized in this investigation. NMR assignments were aided by DEPT and 2D experiments.

cis-1-Azido-4-phenyl-1,2,3,4-tetrahydronaphthalene *cis*-**140a**[31]

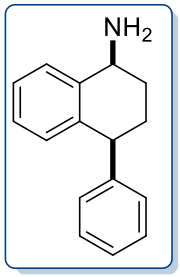


Diphenylphosphoryl azide (1.30 mL, 1.66 g, 6.0 mmol, 1.2 eq.) was added to a solution of *trans*-**137b** (1.131 g, 5.0 mmol) in dry toluene (12.5 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.90 mL, 0.918 g, 6.0 mmol, 1.2 eq.) was added dropwise to the stirring solution. The solution was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in DCM/hexane (1/1) and filtered through a short silica gel pad (approx. 150 mL solvent used), and the solvent was removed in vacuo to give the crude product as a brown oil (4.01 g). The product was purified by column chromatography using hexane/ethyl acetate (95/5) as eluent to give the pure product *cis*-**140a** (966 mg, 77%) as a yellow solid (m.p.: 80–82 °C).

ν_{max} (ATR) 2943 (CH), 2091 (N_3), 1486, 755, 699 cm^{-1}; δ_H (300 MHz) 1.85–2.32 [m, 4H, C(2)H_2 and C(3)H_2], 4.04 [t, 1H, *J* = 6.7, C(4)H], 4.64 [t, 1H, *J* = 4.7, C(1)H], 6.89 (d, 1H, *J* = 7.7, ArH), 7.03–7.42 (m, 8H, ArH) ppm; δ_C (75.5 MHz) 28.1 (CH_2), 28.9 (CH_2), 45.6 [C(4)H], 59.7 [C(1)H], 126.4, 126.5, 128.5, 128.8, 129.3, 130.4 (6 × signals for 7 × aromatic CH), 133.9, 140.2, 146.3 (3 × aromatic C) ppm; elemental analysis: (Found C 77.06; H 6.13; N 16.55. $C_{16}H_{15}N_3$ requires C 77.08; H 6.06; N 16.85%).

This compound is novel and has been fully characterized in this investigation.

cis-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-amine *cis*-**93a**[32]

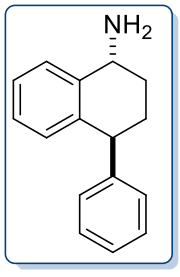


Polystyrene-bound triphenyl phosphine [4.296 g (1.4–2.0 mmol/g loading), ~7.3 mmol, 2.0 eq.] was added to a solution of *cis*-**130a** (0.854 g, 3.4 mmol) in dry THF (50 mL) and stirred for 16 h. Water (10 mL) was added and the reaction solution stirred for an additional 4 h. The reaction mixture was filtered and extracted with DCM (3 × 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give a mixture of the azide *cis*-**130a** and the amine *cis*-**93a**. The reaction product and the polymer-bound catalyst were resuspended in THF (50 mL) and water (5 mL) and stirred for 4d, after which *cis*-**130a** was still evident by IR. The reaction solution was heated to reflux for 2d and worked up as previously described to give the crude amine *cis*-**93a** and unreacted azide *cis*-**130a** as an orange oil. The mixture was dissolved in DCM and aq. HCl (5M) was added until pH 1. The mixture was extracted with DCM and the residue evaporated to give the azide *cis*-**130a**. The aqueous layer was adjusted to pH > 9, extracted with DCM (3 × 10 mL) and evaporated to give the *cis*-amine *cis*-**93a** as a brown oil (0.070 g, 9%).

ν_{max} (ATR) 3279, 2928, 1489, 1447, 759, 726, 700 cm^{-1}; δ_H (300 MHz) 1.66 (br s, 2H, NH_2), 1.75–1.88 [m, 1H, one of C(2)H_2], 1.92–2.22 [m, 3H, one of C(2)H_2 and C(3)H_2], 3.99–4.11 [m, 2H, C(1)H and C(4)H], 6.84 (d, 1H, *J* = 7.7, ArH), 7.00–7.35 (m, 7H, ArH), 7.43 (d, 1H, *J* = 7.0, ArH) ppm; δ_C (75.5 MHz) 28.9 (CH_2), 30.9 (CH_2), 45.8 [C(4)H], 49.4 [C(1)H], 126.1, 126.5, 126.8, 128.2, 128.3, 128.9, 130.0 (7 × aromatic CH), 139.0, 141.6, 146.9 (3 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 224.1434 (calculated 224.1443); enantiomers separated using Chiralcel AS-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL min^{-1}], R_t = 10.7, R_t = 11.5 min, R_t (ketone **134**, no separation) = 14.6 min.

This compound is novel and has been fully characterized in this investigation.

trans-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-amine *trans*-**93b**



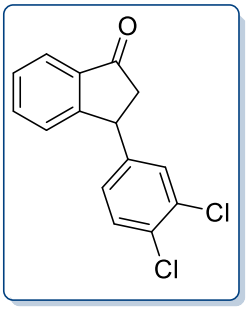
Triphenylphosphine **145** (555 mg, 2.1 mmol, 1.1 eq.) and water (68.4 µL, 2.0 eq.) were added to a stirring solution of *trans*-**140b** (479 mg, 1.9 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled, and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *trans*-**93b** and triphenylphosphine oxide **146** (1.253 g). The residue was suspended in diethyl ether (20 mL) and the insoluble triphenylphosphine oxide by-product

was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM (10 mL) and aq. HCl (5 M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL), the pH was adjusted to 14 with aq. NaOH (5M), and the solution stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the product *trans*-**93b** as a yellow oil (313 mg, 52%).

ν_{max} (ATR) 3357, 2925, 1491, 1450, 749, 700 cm^{-1}; δ_H (300 MHz) 1.57–1.73 (m, 1H, one of C(2)H_2), 1.79–1.95 [m, 1H, one of C(3)H_2], 2.09–2.20 [m, 1H, one of C(2)H_2], 2.24–2.37 [m, 1H, one of C(3)H_2], 4.11 [t, 1H, *J* = 6.1, C(1)H], 4.15 [t, 1H, *J* = 6.8, C(4)H], 6.85 (d, 1H, *J* = 7.8, ArH), 7.05 (d, 2H, *J* = 7.1, ArH), 7.10 (d, 1H, *J* = 7.3, ArH), 7.15–7.35 (m, 4H, ArH), 7.51 (d, 1H, *J* = 7.7, ArH) ppm; δ_C (75.5 MHz) 30.1 [C(3)H_2], 31.8 [C(2)H_2], 45.7 [C(4)H], 49.8 [C(1)H], 126.1, 126.5, 126.7, 127.5, 128.3, 128.7, 130.2 (7 × aromatic CH), 139.0, 147.0 (3 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 224.1435 (calculated 224.1434); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL min^{-1}], R_t = 17.7, R_t = 23.1 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments and by comparison to the related compounds ketone **134**, alcohol *trans*-**137b**, and azide *trans*-**140b**.

3-(3,4-Dichlorophenyl)-2,3-dihydro-*1H*-inden-1-one **135**[33]



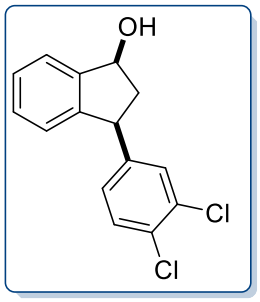
Triflic acid (50 g, 333.0 mmol) was added slowly to a stirring solution of *trans*-cinnamic acid **20** (7.60 g, 51.0 mmol) and dichlorobenzene **110** (35 mL, 45.5 g, 310.0 mmol) at 0 °C. The reaction solution was heated to reflux and stirred for 3d. The reaction solution was cooled and poured onto a mixture of water and ice (70 mL) to quench, stirred for approx. 1 h, and the layers separated. The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 50 mL), water (100 mL) and concentrated to give the crude product **135** as a solution in dichlorobenzene. The dichlorobenzene was removed by vacuum distillation (b.p. 106–108 °C, at unknown pressure), giving the crude product as an orange solid which was recrystallized

from DCM/hexane to give the pure product **135** (8.908 g, 63%) as a white solid m.p. 110–112 °C, lit.: 113–115 °C.[34]

ν_{max} (ATR) 2919, 1698 (C=O), 762 cm^{-1}; δ_H (300 MHz) 2.62 [dd, 1H, *J* = 19.2, 3.9, one of C(2)H_2, 3.23 [dd, 1H, *J* = 19.2, 8.2, one of C(2)H_2,], 4.55 [dd, 1H, *J* = 8.2, 3.9, C(3)H], 6.95 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.20–7.29 (m, 2H, ArH), 7.38 (d, 1H, *J* = 8.3, ArH), 7.41–7.53 (m, 1H, ArH), 7.61 (td,1H, *J* = 7.5, 1.3, ArH), 7.77–7.88 (m, 1H, ArH) ppm; δ_C (75.5 MHz) 43.6 [C(3)H], 46.5 [C(2)H_2], 123.7, 126.7, 127.0, 128.4, 129.7, 130.9 (6 × aromatic CH), 131.2, 133.0 (2 × aromatic C), 135.4 (aromatic CH), 136.8, 144.0, 156.5 (3 × aromatic C), 204.9 (C=O) ppm; enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL min^{-1}], R_t = 34.9, R_t = 37.8 min and using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL min^{-1}], R_t = 35.3, R_t = 38.4 min.

All data is in agreement with previously reported data.[35,36]

cis-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *cis*-**138a**[28,34,35]

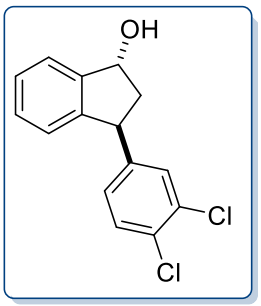


Ketone **135** (8.908 g, 32.1 mmol) in THF (100 mL) was cooled to −15 °C using a salt/ice bath. A solution of sodium borohydride (2.450 g, 64.8 mmol, 2.0 eq.) in water (10 mL) was slowly added to the stirring solution, maintaining the temperature below 0 °C. When the addition was complete the reaction solution was allowed warm to room temperature and stirred for 3 h. The solution was diluted with ice-water (50 mL) and stirred for 1 h. The THF was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water (2 × 50 mL) and brine (1 × 75 mL) and concentrated to give the crude product **138** as a mixture of diastereomers (91:9). The product was purified by column chromatography using diethyl ether/hexane (25/75) as eluent which gave the pure product *cis*-**138a** as a colourless oil (4.975 g, 55%) and the minor diastereomer *trans*-**138b** as a colourless oil (0.358 g, 4%)

cis-**138a:** ν_{max} (ATR) 3306 (OH), 1468, 1030, 756, 742 cm^{-1}; δ_H (300 MHz) 1.89 [ddd, 1H, *J* = 13.1, 8.9, 7.3, one of C(2)H_2], 1.99 (d, 1H, *J* = 7.0, OH), 3.01 [ddd, 1H, *J* = 13.1, 7.6, 7.0, one of C(2)H_2], 4.15 [dd appears as t, 1H, *J* = 8.2, C(3)H], 5.30 [m, 1H, C(1)H], 6.94 (d, 1H, *J* = 7.7, ArH), 7.07 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.22–7.36 (m, 3H, ArH), 7.38 (d, 1H, *J* = 8.3, ArH), 7.48 (d, 1H, *J* =7.4, ArH) ppm; δ_C (75.5 MHz) 46.7 [C(2)H_2], 47.6 [C(3)H], 74.9 [C(1)H], 123.9, 124.9, 127.67,

127.68, 128.7, 130.2, 130.55 (7 × aromatic CH) 130.59, 132.6, 144.4, 144.7, 145.2 (5 × aromatic C) ppm.

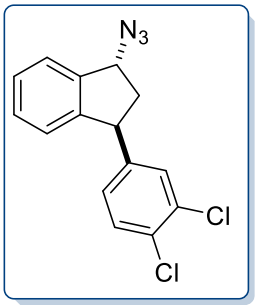
All data is in agreement with previously reported data.[33]



trans-**138b:** ν_{max} (ATR) 3306 (OH), 1469, 1032, 755 cm^{-1}; δ_H (300 MHz) 2.24–2.40 [1H, m, one of C(2)H_2], 2.45–2.63 [1H, m, 7.7, 2.9, one of C(2)H_2], 4.59 [1H, t, *J* = 7.4, C(3)H], 5.38 [1H, dd, *J* = 6.2, 2.7, C(1)H], 6.96 (1H, dd, *J* = 8.3, 2.1, ArH), 6.98–7.05 (1H, m, ArH), 7.21 (1H, d, *J* = 2.1, ArH), 7.27–7.39 (3H, m, ArH), 7.44–7.53 (1H, m, ArH) ppm; δ_C (75.5 MHz) 46.3 [C(2)H_2], 48.2 (CH), 75.1 (CH), 124.6, 125.3, 127.4, 127.8, 129.3, 129.8 (6 × aromatic CH), 130.5 (aromatic C), 130.6 (aromatic CH), 132.6, 144.9, 145.1, 145.6 (4 × aromatic C) ppm.

All data is in agreement with previously reported data.[33]

trans-1-Azido-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-indene *trans*-**141b**

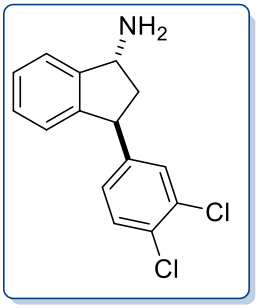


Diphenylphosphoryl azide (0.92 mL, 1.178 g, 4.3 mmol, 1.2 eq.) was added to a stirring solution of *cis*-**138a** (0.99 g, 3.5 mmol) in dry toluene (4 mL) under a nitrogen flow. The mixture was cooled to 0 °C, stirred for 10 mins, and DBU (0.64 mL, 4.3 mmol, 1.2 eq.) was added slowly. The mixture was stirred for 2 h at 0 °C and 16 h at room temperature. The solvent was removed, and the residue passed through a column of silica gel using hexane/ethyl acetate (95/5) as eluent to give the pure product *trans*-**141b** as an orange oil (0.928 g, 86%).

ν_{max} (ATR) 2935, 2092 (N_3), 1474, 1237, 757 cm^{-1}; δ_H (300 MHz) 2.30 [ddd, 1H, *J* = 13.7, 8.3, 6.8, one of C(2)H_2], 2.61 [ddd, 1H, *J* = 13.7, 7.5, 2.4, one of C(2)H_2], 4.53 [t, 1H, *J* = 7.9, C(3)H], 5.03 [dd, 1H, *J* = 6.8, 2.3, C(1)H], 6.94–7.05 (m, 2H, ArH), 7.23 (d, 1H, *J* = 2.1, ArH), 7.28–7.42 (m, 3H, ArH), 7.42–7.52 (m, 1H, ArH) ppm; δ_C (75.5 MHz) 43.4 [C(2)H_2], 48.5 (CH), 64.7 (CH), 124.8, 125.4, 127.4, 127.8, 129.7, 129.9, 130.7 (7 × aromatic CH), 130.8, 132.7, 140.7, 144.1, 145.8 (5 × aromatic C) ppm.

All data is in agreement with previously reported data.[32]

trans-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-amine *trans*-**94b**



Method 1: Sodium iodide (1.504 g, 10.0 mmol, 12.5 eq.) and cerium chloride heptahydrate (453 mg, 1.2 mmol, 1.5 eq.) were added to a solution of azide *trans*-**141b** (246 mg, 0.8 mmol) in acetonitrile (10 mL). The suspension was stirred at reflux for 24 h. The reaction mixture was cooled, diluted with diethyl ether (25 mL) and washed with water (3 × 25 mL), sat. aq. sodium bicarbonate (2 × 25 mL), sat. aq. sodium thiosulfate (25 mL, decolourised solution), brine (30 mL) and dried (Na_2SO_4) to give the crude mixture, comprising of the unreacted azide *trans*-**141b** and the amine product *trans*-**94b** (242 mg). The residue was treated with aq. HCl (10%, 25 mL), washed with DCM (3 × 25 mL) and treated with aq. NaOH (5M) until pH > 9. The aqueous solution was extracted with DCM (3x20 mL) and evaporated to give the impure amine *trans*-**94b** as a green oil (67 mg, 30%).

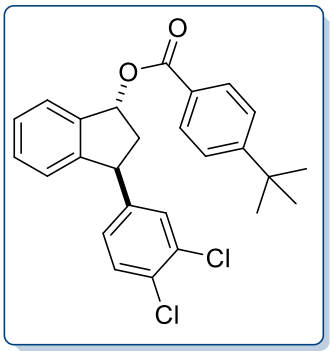
Method 2: Triphenylphosphine **145** (617 mg, 2.45 mmol, 1.1 eq.) and water (77.0 µL, 2.0 eq.) were added to a stirring solution of azide *trans*-**141b** (650 mg, 2.1 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *trans*-**94b** and triphenylphosphine oxide **146**. The residue was suspended in diethyl ether (20 mL) and the insoluble triphenylphosphine oxide by-product **146** was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM and aq. HCl (5M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL) and the pH was adjusted to 14 with aq. NaOH (5M), and the suspension stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the product *trans*-**94b** as a yellow oil (265 mg, 45%).

ν_{max} (ATR) 3364, 3007, 1469, 1264, 732, 703 cm^{-1}; δ_H (300 MHz) 2.22–2.46 [m, 2H, C(2)H_2], 4.51 [dd, 1H, *J* = 8.0, 6.0, C(3)H], 4.57 [dd appears as t, 1H, *J* = 6.0, C(1)H], 6.93 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.02 (d, 1H, *J* = 7.5, ArH), 7.18 (d, 1H, *J* = 2.1, ArH), 7.20–7.37 (m, 3H, ArH), 7.40 (d, 1H, *J* = 7.3, ArH) ppm; δ_C (75.5 MHz) 46.9 [C(2)H_2], 48.2 [C(3)H], 55.8 [C(1)H], 123.9, 125.2, 127.2, 127.7, 128.2, 129.7 (6 × aromatic CH), 130.3 (aromatic C), 130.4 (aromatic CH), 132.5, 144.5, 145.6, 147.4 (4 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 278.0498 (calculated

278.0498); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 95/5, flow rate = 0.5 mL min^{-1}], R_t = 23.1, R_t = 27.1 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments.

trans-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-yl 4-(*tert*-butyl)benzoate *trans*-**144**[37]

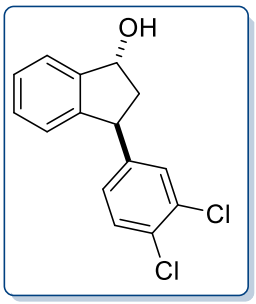


A solution of *cis*-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *cis*-**141a** (1.413 g, 5.1 mmol), triphenylphosphine **145** (2.593 g, 9.9 mmol, ~2.0 eq.), diethyl azodicarboxylate (1.780 g, 10.2 mmol, 2.0 eq.) and 4-*tert*-butylbenzoic acid **143** (1.82 g, 10.2 mmol, 2.0 eq.) in dry THF was stirred overnight at room temperature. The organic solvent was removed to give the crude product as a viscous orange oil. Purification by chromatography on silica gel using diethyl ether/ hexane (4/96) furnished a mixture of the product *trans*-**144** and the benzoic acid **143** as an orange oil. The mixture was dissolved in hexane, stirred with basic alumina for 5 mins, and filtered through a pad of Celite®. The residue was washed with hexane (100 mL), hexane/diethyl ether (99/1) (100 mL) and ethyl acetate (50 mL). All three fractions were free of benzoic acid **143** and were combined to give the product *trans*-**144** as a viscous orange oil (1.295g, 58%).

ν_{max} (ATR) 2964, 1713 (C=O), 1268, 731, 694 cm^{-1}; δ_H (300 MHz) 1.31 [s, 9H, $C(CH_3)_3$], 2.36–2.54 [m, 1H, one of $C(2)H_2$], 2.76 [ddd, 1H, *J* = 14.3, 7.6, 2.3, one of $C(2)H_2$], 4.64 [dd appears as t, 1H, *J* = 7.7, C(3)H], 6.54 [dd, 1H, *J* = 6.5, 2.2, C(1)H], 6.92–7.07 (m, 2H, ArH), 7.24–7.38 (m, 4H, ArH), 7.38–7.48 (m, 2H, *m*-ArH of acid moiety), 7.54–7.66 (m, 1H, ArH), 7.91–8.02 (m, 2H, *o*-ArH of acid moiety) ppm; δ_C (75.5 MHz) 31.2 (3 × CH_3), 35.2 [qC, $C(CH_3)_3$], 43.3 [$C(2)H_2$], 48.7 [C(3)H], 77.5 [C(1)H], 125.1, 125.4, 126.4, 127.5 (4 × aromatic CH), 127.6 (aromatic C), 127.8, 129.8, 130.0 (3 × aromatic CH), 130.7, 132.7 (2 × aromatic C), 133.7, 133.9 (2 × aromatic CH), 141.5, 144.7, 146.9 (3 × aromatic C), 156.8 [aromatic C, **C**$C(CH_3)_3$], 166.5 (C=O) ppm; HRMS (ES^+): $[M+Na]^+$ 461.1040 (calculated 461.1046).

This compound is novel and has been fully characterized in this investigation, data closely aligns with the data for the unsubstituted benzoic acid ester.[37]

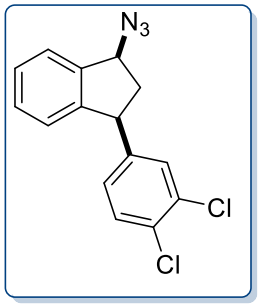
trans-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *trans*-**143b**[37]



Aqueous potassium hydroxide (3M, 8 mL) was added to a stirring solution of *trans*-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-inden-1-yl 4-(*tert*-butyl)benzoate *trans*-**144** (0.430 g, 1.0 mmol) in methanol (16 mL) and THF (16 mL). The reaction mixture was stirred for 3 h. The pH was adjusted to 3 using conc. HCl, followed by addition of water (20 mL). The aqueous layer was extracted with diethyl ether (3 × 20 mL) and the combined organic layers evaporated to give the crude reaction product, a mixture of the benzoic acid **143** and the alcohol *trans*-**138b**. The mixture was suspended in aq. KOH (3M, 10 mL) and stirred for 3 h, the suspension was extracted with diethyl ether (3 × 10 mL), dried, filtered and concentrated to give the product *trans*-**143b** as an orange oil (0.250 g, 92%).

Spectroscopic data were identical to those reported above.

cis-1-Azido-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-indene *cis*-**141a**



Diphenylphosphoryl azide (2.22 mL, 2.84 g, 10.4 mmol, 2.4 eq.)[4] was added to a solution of *trans*-**143b** (1.19 g, 4.3 mmol) in dry toluene (12 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.77 mL, 0.78 g, 5.1 mmol, 1.2 eq.) was added dropwise to the stirring solution. The solution was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed *in vacuo*. The residue was passed through a short column of silica gel using hexane/diethyl ether (95/5) as eluent. The solvent was removed *in vacuo* to give the pure product *cis*-**141a** (691 mg, 53%) as a dark orange oil.

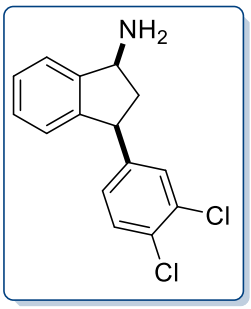
ν_{max} (ATR) 2929, 2090 (C–N_3), 1468, 1254, 758, 747 cm^{-1}; δ_H (300 MHz) 2.02 [ddd, 1H, *J* = 13.3, 8.4, 7.7, one of C(2)H_2], 3.01 [ddd appears as dt, 1H, *J* = 13.3, 7.7, one of C(2)H_2], 4.24 [dd appears as t, 1H, *J* = 8.2, C(3)H], 4.93 [dd appears as t, 1H, *J* = 7.3, C(1)H], 6.97 (d, 1H, *J* = 7.3, ArH), 7.05 (dd, 1H, *J* = 8.2, 2.1, ArH), 7.26–7.52 (m, 5H, ArH) ppm; δ_C (75.5 MHz) 42.6 [C(2)H_2], 48.1 [C(3)H], 64.3 [C(1)H], 124.3, 125.2, 127.6, 127.9, 129.2, 130.2, 130.7 (7 × aromatic CH),

[4] Due to error, twice the required amount of DPPA was added during this reaction. The initial purification using ethyl acetate and hexane (95/5) as eluent resulted in coelution with the DPPA. Switching to a less polar solvent system (as above) was more effective. This is discussed in the relevant section in chapter 5.

130.9, 132.7, 141.2, 144.2, 144.6 (5 × aromatic C) ppm; HRMS (ES^+): $[M-N_3]^+$ 261.0237 (calculated 261.0232).

This compound is novel and has been fully characterized in this investigation.

cis-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-amine *cis*-94a



Triphenylphosphine **145** (658 mg, 2.5 mmol, 1.2 eq.) and water (81.8 µL, 2.0 eq.) were added to a stirring solution of azide *cis*-**141b** (691 mg, 2.3 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *cis*-**94a** and triphenylphosphine oxide **146**. The residue was suspended in diethyl ether (30 mL) and the insoluble triphenylphosphine oxide by-product **146** was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM and aq. HCl (5M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL) and the pH was adjusted to 14 with aq. NaOH (5M), and the mixture stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the product *cis*-**94a** as a yellow oil (313 mg, 52%).

ν_{max} (ATR) 3372, 2957, 1468, 762, 731 cm^{-1}; δ_H (300 MHz) 1.67 [ddd, 1H, *J* = 12.4, 10.6, 9.5, one of C(2)H_2], 2.94 [ddd appears as dt, 1H, *J* = 12.4, 7.0, one of C(2)H_2], 4.14 [dd, 1H, *J* = 10.6, 7.3, C(3)H], 4.37 [dd, 1H, *J* = 9.0, 7.2, C(1)H], 6.88 (d, 1H, *J* = 7.4, ArH), 7.07 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.16–7.50 (m, 5H, ArH) ppm; δ_C (75.5 MHz) 48.0 [C(3)H], 48.7 [C(2)H_2], 55.9 [C(1)H], 123.2, 124.6 (2 × aromatic CH), 127.3 (aromatic C), 127.4, 127.7, 127.8 (3 × aromatic CH), 128.6 (aromatic C), 130.3, 130.5 (2 × aromatic CH), 132.0, 132.5, 144.6 (3 × aromatic C) ppm; HRMS (ES^+): $[M+H]^+$ 278.0504 (calculated 278.0498); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 95/5, flow rate = 0.5 mL min^{-1}], R_t = 16.5, R_t = 19.2 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments.

Procedures for biotransformations

General procedure for oxidative deamination reactions

E. Coli cells containing overexpressed transaminase (30 mg or 50 mg, as indicated in the appropriate tables) were suspended in 50 mM sodium phosphate buffer (pH 8.5, 400 µL) in a 15 mL centrifuge tube, the suspension was sonicated if required using a probe for 10 s, followed by 30 s on ice, this was repeated five times to lyse the cells. PLP solution (100 µL buffer, overall conc. 1mM) and sodium pyruvate (in 100 µL buffer, overall 1 eq.) solutions were added, followed by the amine substrate (20 mM or 50 mM, as indicated, in 100 µL DMSO), any additional buffer or DMSO was added as required to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 16 h or 24 h as indicated in the corresponding tables. The reaction was stopped through the addition of aqueous NaOH solution (5M, 400 µL). Ethyl acetate (≈ 4 mL) was added and the tubes were centrifuged to pellet the cells. The organic phase was passed through a plug of Na_2SO_4 and the solvent was removed *in vacuo*. The crude products were analysed by ^{1}H NMR and chiral HPLC, except when amine **120** was used as substrate (Table 5.3, Entry 12), in this case the reaction mixture containing ketone **159** and amine **120** was derivatized (procedure for preparation of **158**) before chiral HPLC analysis (conditions in Appendix I).

Results are shown in Table 5.3, Table 5.4, Table 5.9, Table 5.10, Table 5.11.

General procedure for reductive amination reactions

*Using isopropylamine **97** as amine donor:* *E. Coli* cells containing overexpressed CV-TA (30 mg) were suspended in 1M isopropylamine solution (pH adjusted to 10 using conc. HCl), in a 15 mL centrifuge tube. PLP was solution (in 100 µL of ^{i}PrNH$_2$ solution, overall conc. 1 mM), substrate (in 100 µL DMSO, overall conc. 20 mM or 50 mM) solutions were added, additional buffer was added to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

The results are shown in Table 5.5.

Using AlaDH system:[38] *E. Coli* cells containing overexpressed transaminase (50 mg) were suspended in 50 mM sodium phosphate buffer (pH 7.5, 400 µL) in a 15 mL centrifuge tube. PLP solution (100 µL buffer, overall conc. 1 mM), L-alanine (in 100 µL buffer, overall 5.0 eq.), NAD^+ (in 100 µL buffer, overall 1 mM), ammonium formate (in 100 µL buffer, overall 150 mM), substrate (in 100 µL DMSO, overall conc. 50 mM) solutions were added, followed by the auxiliary enzymes formate dehydrogenase (11 U), alanine dehydrogenase (12 U), additional

buffer was added to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

Results are shown in Table 5.6.

Using the LDH system:[38] *E. Coli* cells containing overexpressed transaminase (50 mg) were suspended in 50 mM sodium phosphate buffer (pH 7.5, 400 µL) in a 15 mL centrifuge tube. PLP solution (100 µL buffer, overall conc. 1 mM), L-alanine (in 100 µL buffer, overall 5.0 eq.), NAD^+ (in 100 µL buffer, overall 1 mM), glucose (in 100 µL buffer, overall 150 mM), substrate (in 100 µL DMSO, overall conc. 50 mM) solutions were added, followed by the auxiliary enzymes lactate dehydrogenase (90 U), glucose dehydrogenase (30 U). The reaction mixture as shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

Results are shown in Table 5.7.

Use of alternative amine sources:[39] *E. Coli* cells containing overexpressed CV-TA (30 mg) were suspended in sodium phosphate buffer (pH 8.5, 50 mM), in a 15 mL centrifuge tube. PLP was added (in 100 µL of buffer, overall conc. 1 mM), ketone **148** (in 100 µL DMSO, overall conc. 50 mM) and amine donor (added neat or in 100 µL buffer, overall conc 250 mM) solutions were added. The solution was shaken at 30 °C, 400 rpm for 24 h. The reactions were stopped, worked up and analysed as above.

Results are shown in Table 5.8.

6.6 References

1. Cipiciani, A.; Fringuelli, F.; Maria Scappini, A. *Tetrahedron* 1996; *52*:9869-76.
2. Corberan, R.; Mszar, N. W.; Hoveyda, A. H. *Angew. Chem. Int. Ed.* 2011; *50*:7079-82.
3. Mino, T.; Hasegawa, T.; Shirae, Y.; Sakamoto, M.; Fujita, T. *J. Organomet. Chem.* 2007; *692*:4389-96.
4. Adam, W.; Bosio, S. G.; Turro, N. J.; Wolff, B. T. *J. Org. Chem.* 2004; *69*:1704-15.
5. Ramage, R.; Blake, A. J.; Florence, M. R.; Gray, T.; Raphy, G.; Roach, P. L. *Tetrahedron* 1991; *47*:8001-24.
6. Rioz-Martinez, A.; de Gonzalo, G.; Torres Pazmino, D. E.; Fraaije, M. W.; Gotor, V. *J. Org. Chem.* 2010; *75*:2073-6.
7. Biswas, B.; Sen, P. K.; Venkateswaran, R. V. *Tetrahedron* 2007; *63*:12026-36.
8. Cannone, P.; Bélanger, D.; Lemay, G. *Synthesis* 1980; *1980*:301-3.
9. Liang, H.; Ciufolini, M. A. *Chem. Eur. J.* 2010; *16*:13262-70.
10. Yates, P.; Macas, T. S. *Can. J. Chem.* 1988; *66*:1-10.
11. Peters, M.; Trobe, M.; Tan, H.; Kleineweischede, R.; Breinbauer, R. *Chem. Eur. J.* 2013; *19*:2442-9.
12. Kumari, G. N.; Ganesh, M. R.; Anitha, R.; Sivasubramanian, A. *Z. Naturforsch. C.* 2004; *59*:405-7.
13. Dampawan, P.; Zajac, W. W. *J. Org. Chem.* 1982; *47*:1176-81.
14. Ballini, R.; Petrini, M. *Synth. Commun.* 1986; *16*:1781-8.
15. Ranu, B. C.; Das, A. R. *Tetrahedron Lett.* 1992; *33*:2361-2.
16. Wang, C.; Pettman, A.; Basca, J.; Xiao, J. *Angew. Chem. Int. Ed.* 2010; *49*:7548-52.
17. Weiberth, F. J.; Hall, S. S. *J. Org. Chem.* 1986; *51*:5338-41.
18. Miriyala, B.; Bhattacharyya, S.; Williamson, J. S. *Tetrahedron* 2004; *60*:1463-71.
19. Vahermo, M.; Suominen, T.; Leinonen, A.; Yli-Kauhaluoma, J. *Arch. Pharm.* 2009; *342*:201-9.
20. Bondarev, O.; Bruneau, C. *Tetrahedron: Asymmetry* 2010; *21*:1350-4.
21. Liardo, E.; Ríos-Lombardía, N.; Morís, F.; Rebolledo, F.; González-Sabín, J. *ACS Catal.* 2017; *7*:4768-74.
22. Kano, T.; Kobayashi, R.; Maruoka, K. *Org. Lett.* 2016; *18*:276-9.
23. Terrasson, V.; Marque, S.; Georgy, M.; Campagne, J.-M.; Prim, D. *Adv. Synth. Catal.* 2006; *348*:2063-7.
24. Poon, D.; Brinner, K.; Doughan, B. *Synlett* 2009; *2009*:991-3.
25. Prakash, G. K. S.; Yan, P.; Torok, B.; Olah, G. A. *Catal. Lett.* 2003; *87*:109-12.
26. Wei, D.; Li, Y.; Liang, F. *Adv. Synth. Catal.* 2016; *358*:3887-96.
27. Miyano, S.; Tatsuoka, T.; Suzuki, K.; Imao, K.; Satoh, F.; Ishihara, T.; Hirotsu, I.; Kihara, T.; Hatta, M.; Horikawa, Y.; Sumoto, K. *Chem. Pharm. Bull.* 1990; *38*:1570-4.
28. Froimowitz, M.; Wu, K.-M.; Moussa, A.; Haidar, R. M.; Jurayj, J.; George, C.; Gardner, E. L. *J. Med. Chem.* 2000; *43*:4981-92.
29. Kopecky, K. R.; Hall, M. C. *Can. J. Chem.* 1981; *59*:3095-104.
30. Bégué, J.-P.; Cerceau, C.; Dogbeavou, A.; Mathé, L.; Sicsic, S. *J. Chem. Soc., Perkin Trans. 1* 1992; *22*:3141-4.
31. Fernández, R.; Ros, A.; Magriz, A.; Dietrich, H.; Lassaletta, J. M. *Tetrahedron* 2007; *63*:6755-63.
32. Walton, J. G.; Jones, D. C.; Kiuru, P.; Durie, A. J.; Westwood, N. J.; Fairlamb, A. H. *ChemMedChem* 2011; *6*:321-8.
33. Lee, S. H.; Park, S. J.; Kim, I. S.; Jung, Y. H. *Tetrahedron* 2013; *69*:1877-80.
34. Cossy, J.; Belotti, D.; Maguer, A. *Synlett* 2003; *2003*:1515-7.

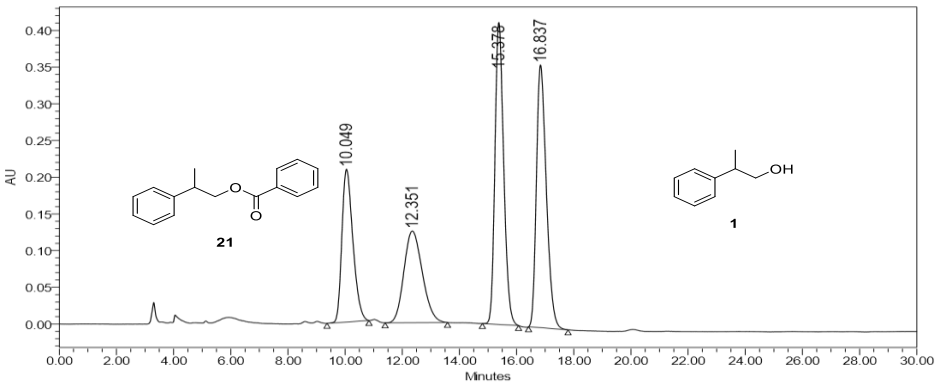
35. Juhl, K.; Nørager, N.; Lorentz-Petersen, L.; Lyngsø, L.; Kehler, J. *Synlett* 2011; *2011*:1753-5.
36. Roesner, S.; Casatejada, J. M.; Elford, T. G.; Sonawane, R. P.; Aggarwal, V. K. *Org. Lett.* 2011; *13*:5740-3.
37. Meltzer, P. C.; Blundell, P.; Wang, P.; Madras, B. K.; 2004; US Patent 20040014992; CAN: 140:128271
38. Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Sattler, J. H.; Kroutil, W. *Adv. Synth. Catal.* 2011; *353*:3227-33.
39. Galman, J. L.; Slabu, I.; Weise, N. J.; Iglesias, C.; Parmeggiani, F.; Lloyd, R. C.; Turner, N. J. *Green Chem.* 2017; *19*:361-6.

Appendices

Appendix I: Chiral HPLC Conditions

Chapter 2

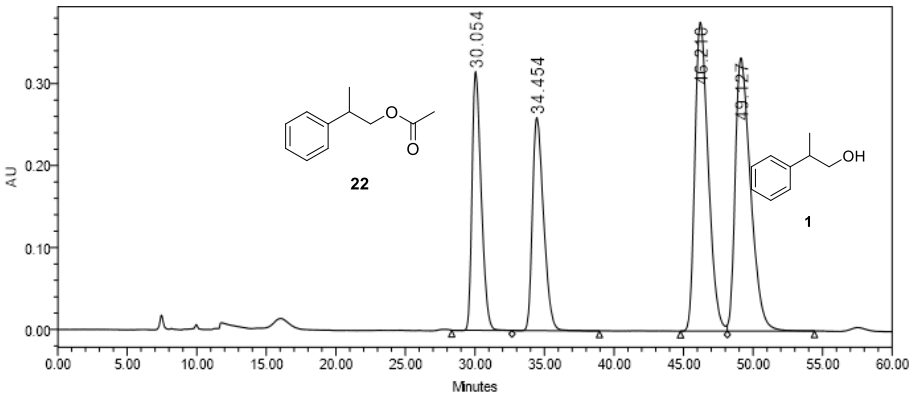
Table A1



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OB-H	1	209.8	99/1	25	*(R)*-**21**	10.0
					(S)-**21**	12.4
					(S)-**1**	15.4
					(R)-**1**	16.8

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

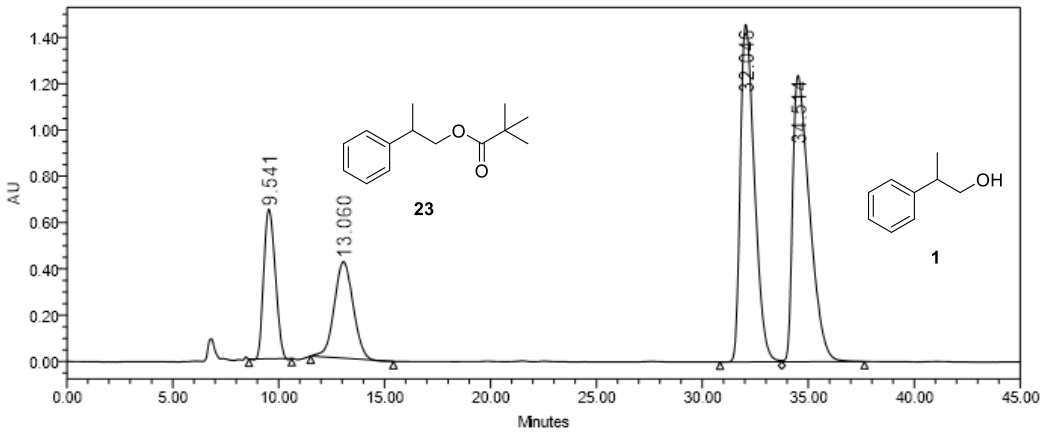
Table A2



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OB-H	0.5	209.8	99.5/0.5	25	*(R)*-**22**	30.1
					(S)-**22**	34.5
					(S)-**1**	46.2
					(R)-**1**	49.1

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

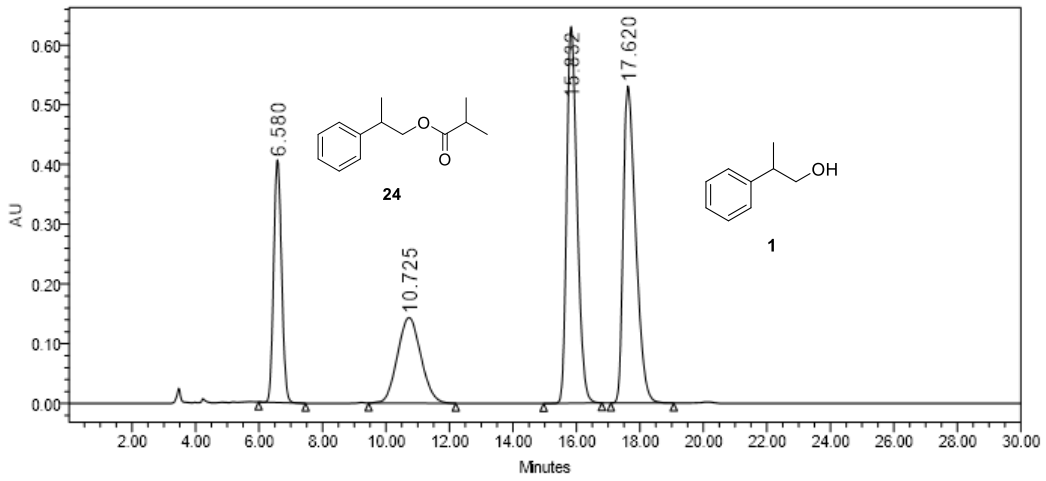
Table A3



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OB-H	0.5	209.8	99/1	25	*(S)*-**23**	9.5
					(R)-**23**	13.1
					(S)-**1**	32.0
					(R)-**1**	34.5

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

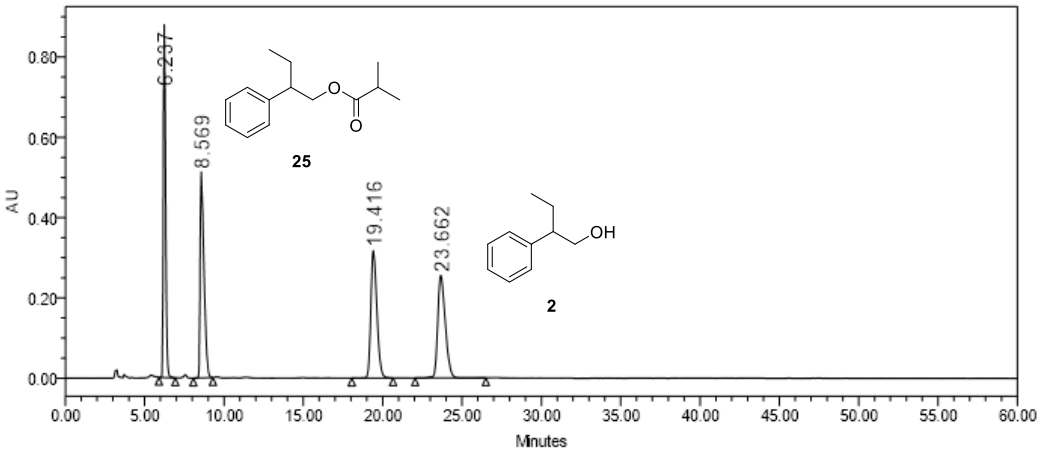
Table A4



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OB-H	0.5	209.8	99/1	25	*(R)*-**24**	6.6
					(S)-**24**	10.7
					(S)-**1**	15.8
					(R)-**1**	17.6

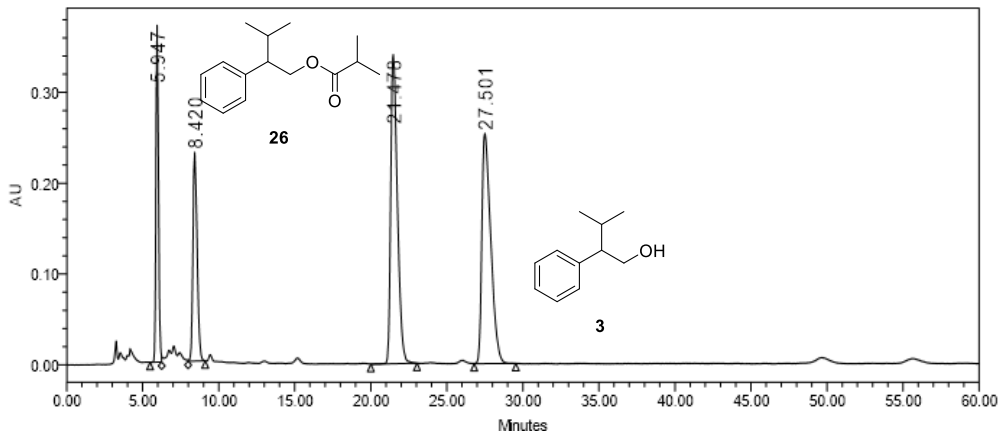
The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

Table A5



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OJ-H	1	209.8	98/2	25	**25**	6.2
					25	8.6
					2	19.4
					2	23.7

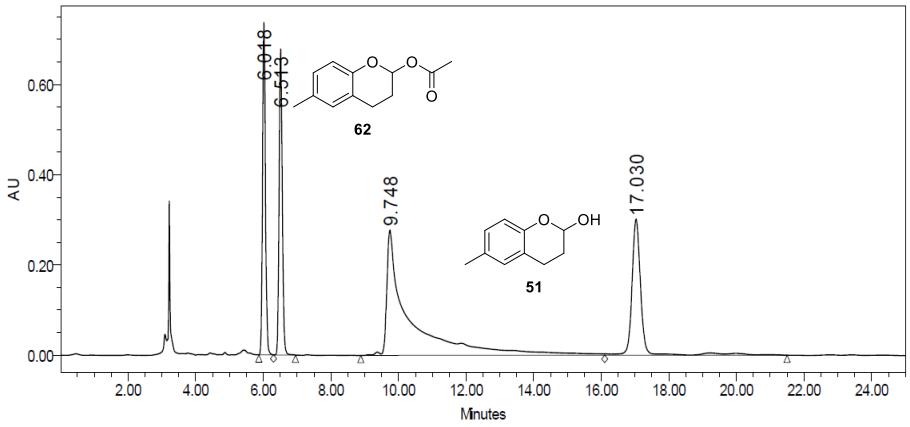
Table A6



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Chiralcel OJ-H	1	209.8	99/1	25	**26**	5.9
					26	8.4
					3	21.5
					3	27.5

Chapter 3

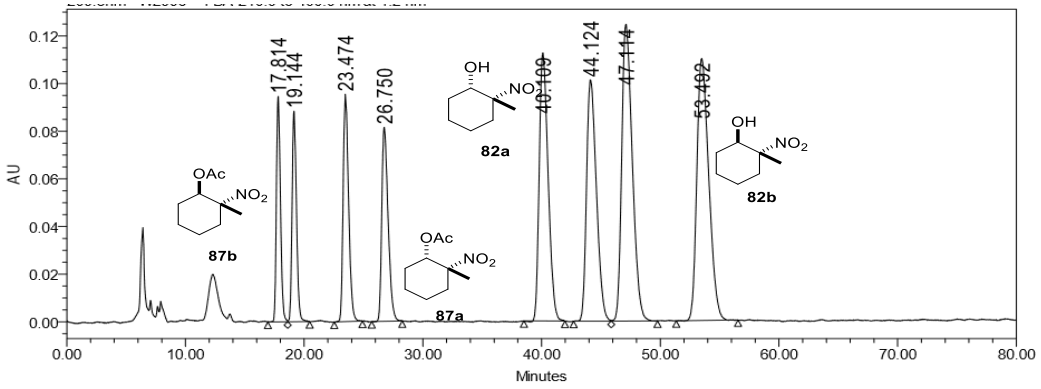
Table A7



Column	Flow (mL/ min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time (min)
Lux Cellulose 4	1	209.8	95/5	25	(–)-**62**	6.0
					(+)-**62**	6.5
					51	9.7
					51	17.0

Chapter 4

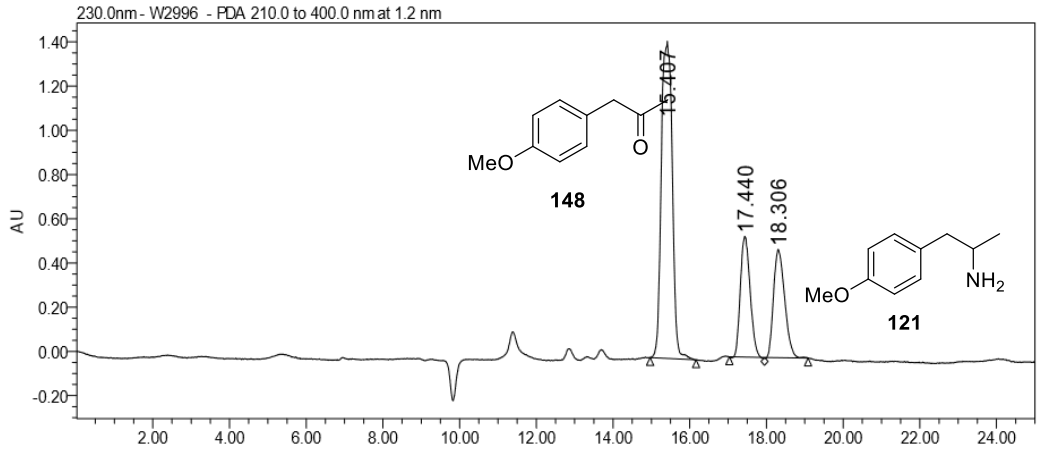
Table A8



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	210	98.5/1.5	25	**87b**	17.8 19.1
					87a	23.5 26.8
					82a	40.1 44.1
					82b	47.1 53.4

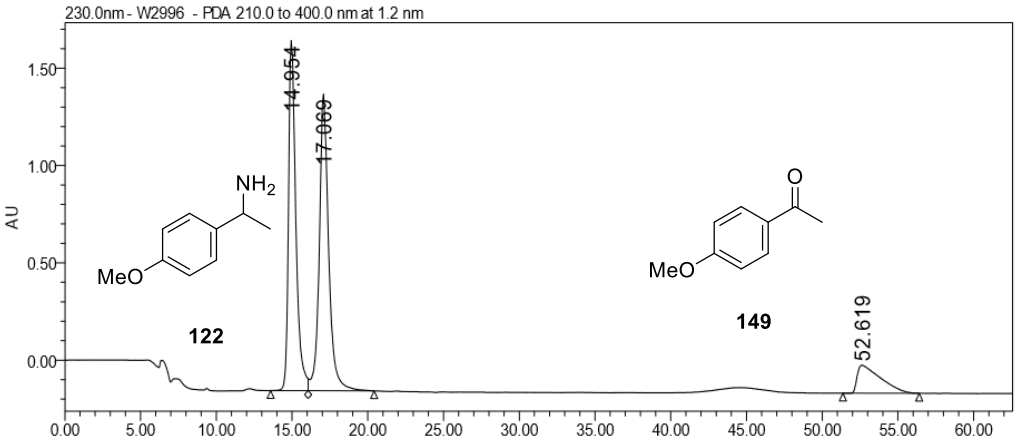
Chapter 5

Table A9



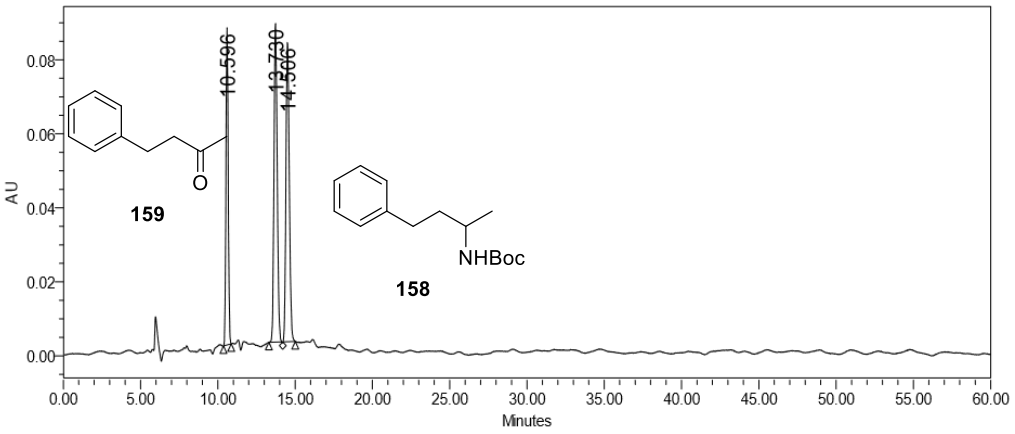
Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Lux Amylose 1	0.4	230	90/10* (*contains 1% v/v DEA)	25	**148**	15.4
					121	17.4
					121	18.3

Table A10



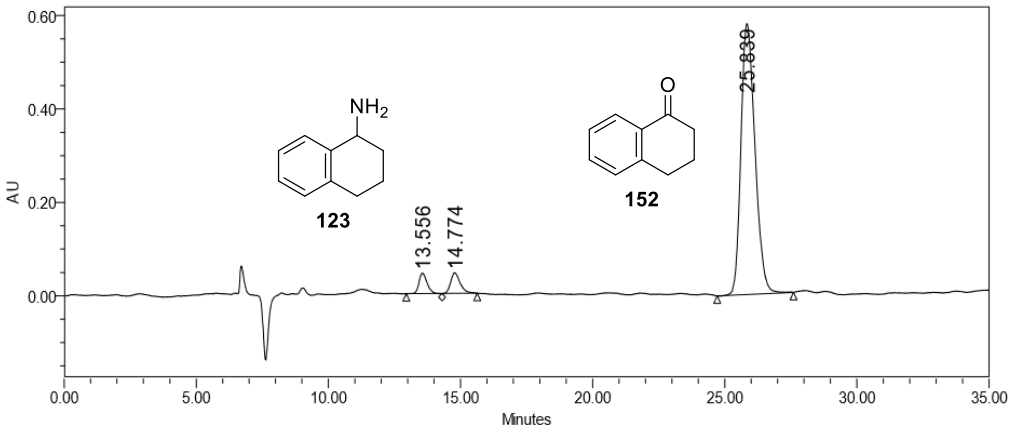
Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OB-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	**122**	15.0
					122	17.1
					149	52.6

Table A11



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time
Lux Amylose 1	0.5	260	95/5* (*contains 1% v/v DEA)	25	**159**	10.6
					158	13.7
					158	14.5

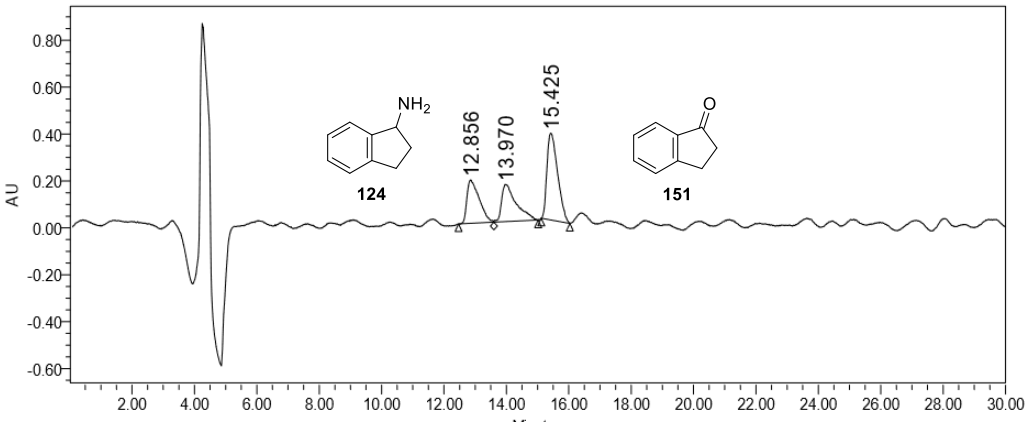
Table A12



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OB-H	0.5	230	95/5* (*contains 1% v/v DEA)	25	*(R)*-**123**	13.6
					(S)-**123**	14.8
					152	25.8

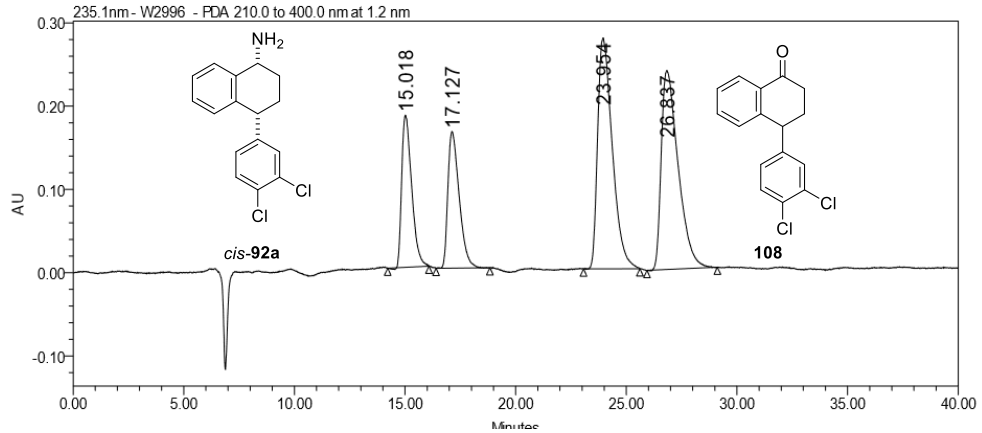
The absolute stereochemistry was assigned by comparison to an enantiopure sample of *(S)*-**123** obtained from Sigma Aldrich

Table A13



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	1	215	99/1* (*contains 1% v/v DEA)	25	**124**	12.9
					124	14.0
					151	15.4

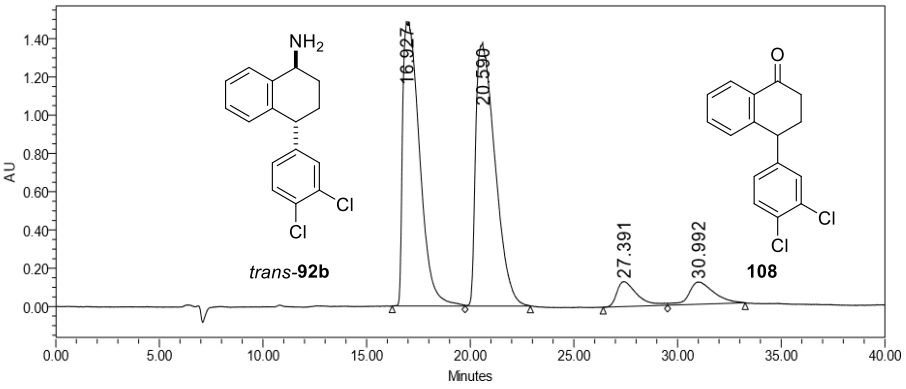
Table A14



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	235	90/10* (*contains 1% v/v DEA)	25	*(1S,4S)*-**92a**	15.0
					(1R,4R)-**92a**	17.1
					(4R)-**108**	24.0
					(4S)-**108**	26.8

The absolute stereochemistry was assigned by comparison to the enantiopure ketone (*4S*)-**108**, which was donated by Pfizer.

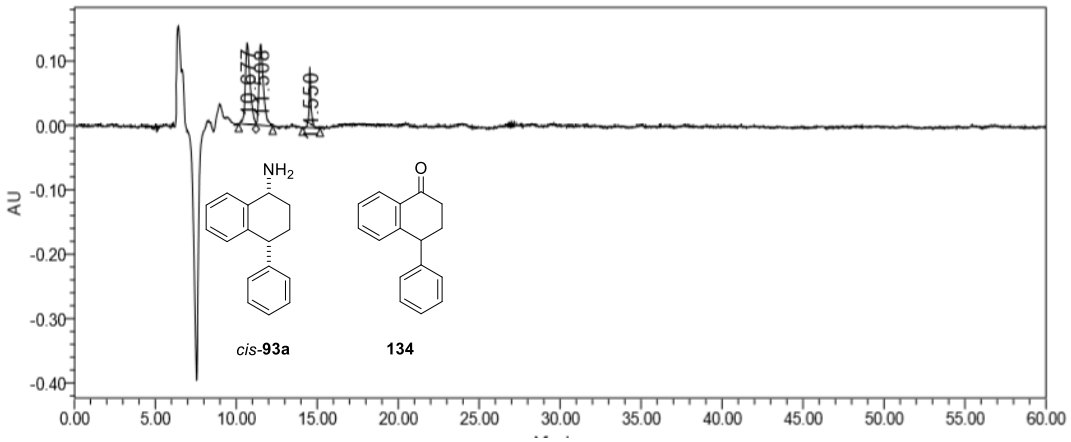
Table A15



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	*(1S,4R)*-**92b**	16.9
					(1R,4S)-**92b**	20.6
					(4R)-**108**	27.4
					(4S)-**108**	31.0

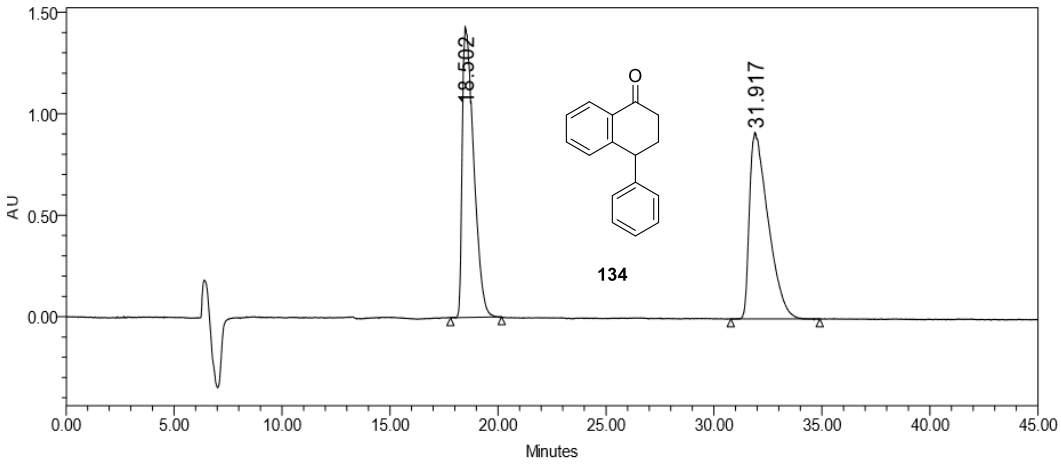
The absolute stereochemistry was assigned by comparison to the enantiopure ketone (*4S*)-**108**, which was donated by Pfizer.

Table A16



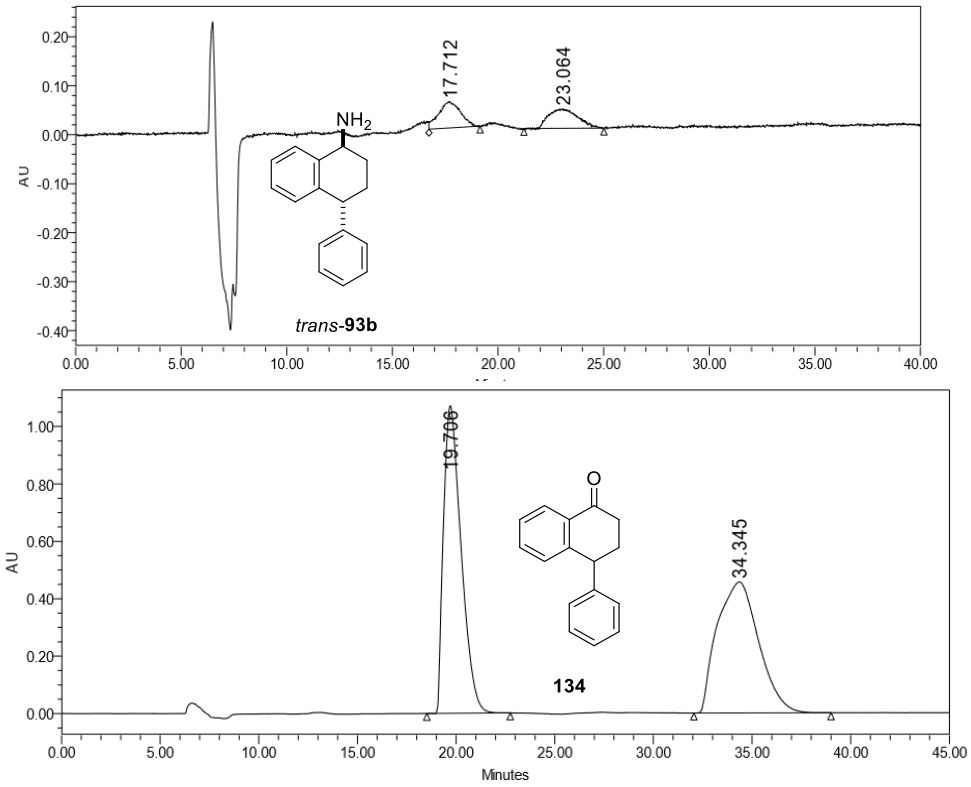
Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel AS-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	**93a**	10.7
					93a	11.5
					134	14.6

Table A17



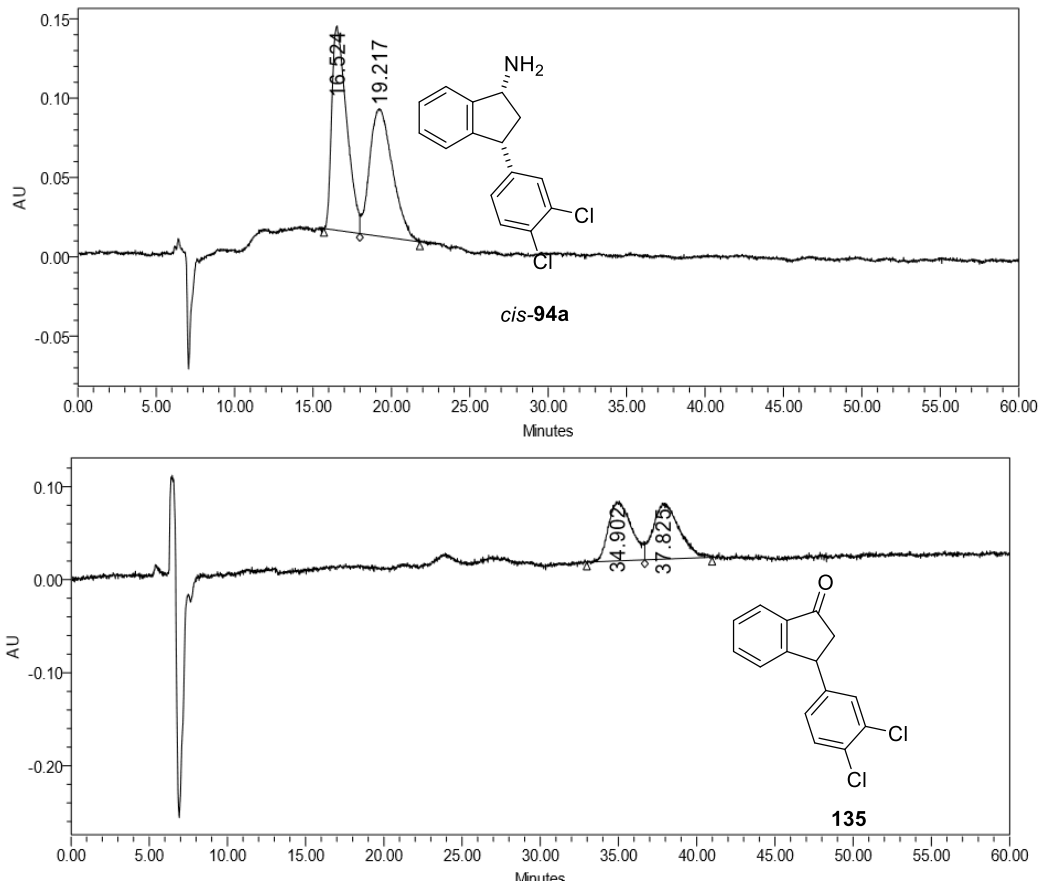
Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	**134**	18.5
					134	31.9

Table A18



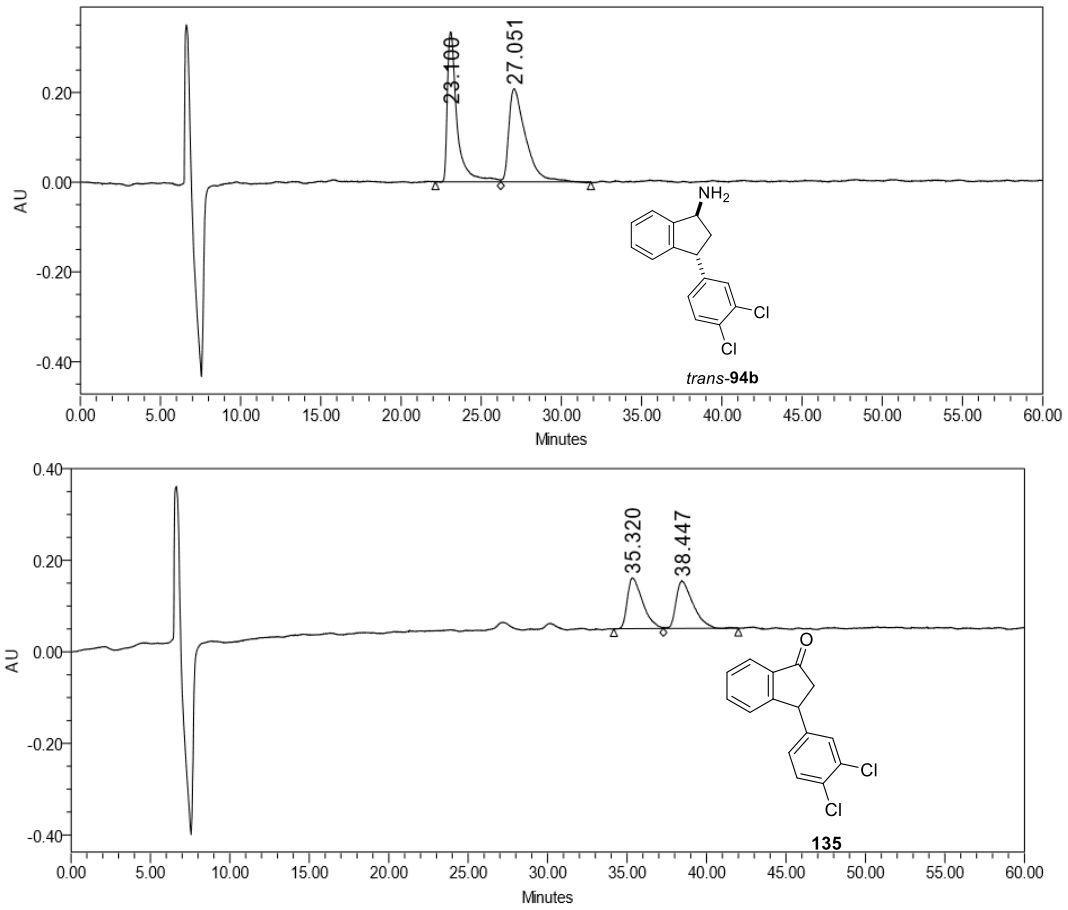
Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	**93b**	17.7
					134	19.7
					93b	23.1
					134	34.3

Table A19



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OB-H	0.5	230	95/5* (*contains 1% v/v DEA)	25	**94a**	16.5
					94a	19.2
					135	34.9
					135	37.8

Table A20



Column	Flow (mL/min)	λ (nm)	Mobile Phase (*n*-hexane/ ^{i}PrOH)	Temp (°C)	Compound	Retention Time
Chiralcel OJ-H	0.5	230	90/10* (*contains 1% v/v DEA)	25	**94b**	23.1
					94b	27.1
					135	35.3
					135	38.4

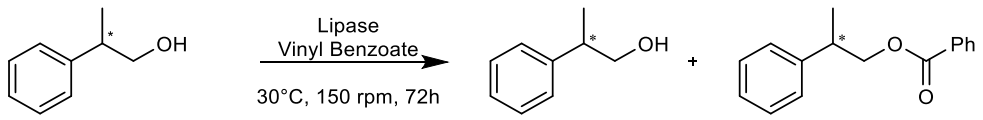
Appendix II: List of Abbreviations

Ac_2O	acetic anhydride
ADH	alcohol dehydrogenase
AlaDH	alanine dehydrogenase
API	active pharmaceutical ingredient
approx.	approximately
aq.	aqueous
Ar	aryl
ATA	amine transaminase
atm	atmospheres
b.p.	boiling point
br s	broad singlet
CAL-B	*Candida antarctica* lipase B (immobilised)
conv.	Conversion
CV	*Chromobacterium violaceum*
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
de	diastereomeric excess
DEA	diethylamine
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement of polarisation transfer
DIP-Cl	B-Chlorodiisopinocamphenylborane
DKR	dynamic kinetic resolution
DMAP	*N*,*N*-dimethylaminopyridine
DMF	dimethyl formamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
dt	doublet of triplets
E. coli	*Escherichia coli*
ee	enantiomeric excess
eq.	equivalents
ERED	ene-reductase
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
EtONa	sodium ethoxide
FDH	formate dehydrogenase
g	gram
GDH	glucose dehydrogenase
Gl	glycine
h	hour
HHDH	halohydrin dehalogenase
His	histidine
HMBC	heteronuclear multiple bond correlation
HNL	hydroxynitrile lyase
HPLC	high performance liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
Immob.	Immobilised
IPA	isopropanol
$^{i}PrNH_2$	isopropylamine

^{i}PrOH	isopropanol
^{i}Pr	isopropyl
IRED	imine reductase
KOH	potassium hydroxide
KR	kinetic resolution
KRED	ketoreductase
LDH	lactate dehydrogenase
m	multiplet
M	molar
Me	methyl
MeOH	methanol
mg	milligram
min	minute
mL	millilitre
mmol	millimole
mol	mole
m.p.	melting point
MTBE	methyl *tert*-butyl ether
$NaBH_4$	sodium borohydride
NAD^+	Nicotinamide adenine dinucleotide
NAD(P)H	nicotinamide adenine dinucleotide (phosphate)
NaOH	sodium hydroxide
NEt_3	triethylamine
NMR	nuclear magnetic resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
PCC	pyridinium chlorochromate
PDA	photodiode array
Ph	phenyl
PLP	pyroxidal 5′-phosphate
PMP	pyroxamine 5′-phosphate
PPL	porcine pancreatic lipase
ppm	parts per million
psi	pounds per square inch
q	quartet
qd	quartet of doublets
R_f	retention factor
rpm	revolutions per minute
RT	room temperature
s	singlet
sat.	saturated
sept	septet
sym m	symmetrical multiplet
t	triplet
ω-TA	ω-transaminase
TA(m)	transaminase
^{t}Bu	*tert*-butyl
td	triplet of doublets
tdd	triplet of doublet of doublets
TEMPO	(2,2,6,6-tetramethyl-1-piperidin-1-yl)oxyl
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMG	1,1,3,3-tetramethylguanidine
TMS	tetramethylsilane
TPPO	triphenylphosphine oxide
% v/v	volume per volume
% w/w	weight per weight

Appendix III: Additional tables for chapter 2

*Table 21 Supplementary data for the transesterification of **1** using vinyl benzoate*



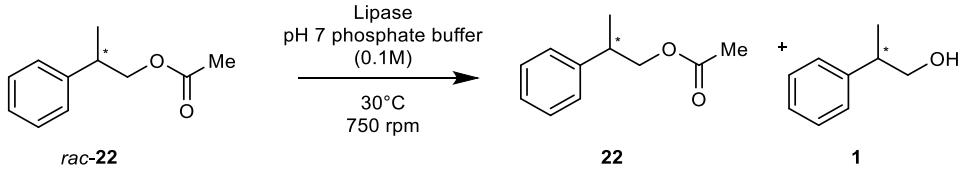
rac-**1** **1** **21**

Enzyme Source	Conversion (%)		*ee* (%)		E
	^{1}H NMR	E_{calc}	ee_s	ee_p	
No enzyme	0	-[a]	-[a]	-[a]	-[a]
Hog Pancreas Lipase	0	-[a]	-[a]	-[a]	-[a]
Candida antarctica Lipase B (immobilised)	0	-[a]	-[a]	-[a]	-[a]
Pseudomonas fluorescens (immobilised)	0	-[a]	-[a]	-[a]	-[a]
Amano PS Lipase	0	-[a]	-[a]	-[a]	-[a]
Lipase from *Candida cylindracea*	100	-[a]	-[a]	-[a]	-[a]

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, E_{calc}, and E were not determined.

Shown below is the data from the time screen for hydrolysis of **22**, supplement to the data in **Table 2.3**, entries marked in blue are also shown in Table 2.3.

Table 22



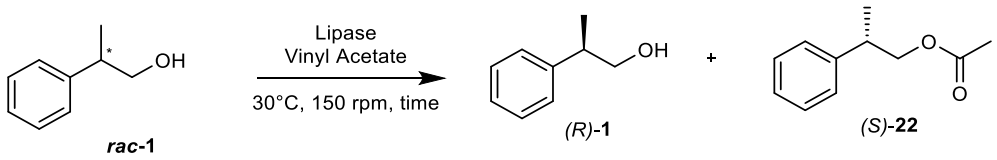
rac-**22** **22** **1**

Lipase Source	Time (h)	Conversion (%)		*ee* (%)		E
		^{1}H NMR	E_{calc}	ee_s	ee_p	
Blank	65	4	-[a]	-[a]	-[a]	-[a]
	48	0	-[a]	-[a]	-[a]	-[a]
	24	0	-[a]	-[a]	-[a]	-[a]
	18	0	-[a]	-[a]	-[a]	-[a]
	6	0	-[a]	-[a]	-[a]	-[a]
Hog pancreas Lipase	65	60	55	54	65	6
	48	56	56	55	71	7
	24	72	72	35	92	6
	18	62	63	46	79	6
	6	19	17	53	11	4
Candida antarctica Lipase B (immobilised)	65	87	62	16	26	2
	48	69	70	38	87	6
	24	55	54	61	72	9
	18	56	47	55	73	7
	6	21	21	70	18	7
Pseudomonas fluorescens (immobilised)	65	87	62	6	10	1
	48	57	59	13	19	2
	24	52	53	15	17	2
Amano PS Lipase	65	100	-[a]	-[a]	-[a]	-[a]
	48	100	-[a]	-[a]	-[a]	-[a]
	24	100	-[a]	-[a]	-[a]	-[a]
	18	100	-[a]	-[a]	-[a]	-[a]
	6	57	57	19	25	2
Lipase from *Candida cylindracea* (gives (R)-1 as product)	65	87	60	2	3	1
	48	56	47	4	4	1
	24	62	58	5	7	1
	18	66	64	4	7	1
	6	42	38	5	3	1

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, E_{calc}, and E were not determined.

Shown below is the data from the time screen for transesterification of **1** with vinyl acetate, supplement to the data in **Table 2.4,** entries marked in blue are also shown in Table 2.4.

*Table 23 Supplemental data for the transesterification of **1***

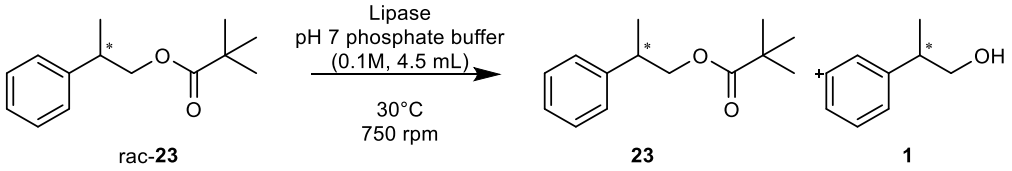


***rac*-1** *(R)*-**1** *(S)*-**22**

Lipase Source	Time (h)	Conversion (%)		*ee* (%)		E
		^{1}H NMR	E_{calc}	ee_s	ee_p	
Blank	24	0	-[a]	-[a]	-[a]	-[a]
	18	0	-[a]	-[a]	-[a]	-[a]
	8	0	-[a]	-[a]	-[a]	-[a]
	6	0	-[a]	-[a]	-[a]	-[a]
	4	0	-[a]	-[a]	-[a]	-[a]
	2	0	-[a]	-[a]	-[a]	-[a]
Hog pancreas lipase	24	78	79	99	27	7
	18	58	47	85	63	11
	8	38	37	45	75	11
	6	41	40	48	73	10
Candida antarctica Lipase B (immobilised)	24	100	-[a]	-[a]	-[a]	-[a]
	18	100	-[a]	-[a]	-[a]	-[a]
	6	93	-[b]	3	0	-[b]
	4	92	-[b]	7	0	-[b]
	2	52	67	2	1	1
Pseudomonas fluorescens (immobilised)	24	99	-[a]	-[a]	-[a]	-[a]
	18	100	-[a]	-[a]	-[a]	-[a]
	6	80	81	14	3	1
	4	72	72	12	4	1
	2	46	43	4	5	1
Amano PS Lipase	24	79	80	45	11	2
	18	65	67	31	15	2
	8	28	23	7	24	2
	6	44	45	16	20	2
	4	-[a]	-[a]	-[a]	-[a]	-[a]
	2	-[a]	-[a]	-[a]	-[a]	-[a]
Lipase from *Candida cylindracea (gives (R)-1 as product)*	24	100	-[a]	-[a]	-[a]	-[a]
	18	99	-[a]	-[a]	-[a]	-[a]
	6	98	-[a]	-[a]	-[a]	-[a]
	4	96	96	45	2	1
	2	58	89	31	4	1

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, E_{calc}, and E were not determined; [b]E_{calc} and E were not determined as this required *ee* >0

*Table 24 Supplemental data for the hydrolysis of **23***



Enzyme Source	Conversion (%)		*ee* (%)		E
	^{1}H NMR	E_{calc}	ee_s	ee_p	
No Lipase	0	-[a]	-[a]	-[a]	-[a]
Hog pancreas Lipase	3	29	4	10 (R)	1
Candida antarctica Lipase B (immobilised)	0	-[a]	-[a]	-[a]	-[a]
Pseudomonas fluorescens (immobilised)	0	-[a]	-[a]	-[a]	-[a]
Amano PS Lipase	15	13	14	90 (S)	22
Lipase from *candida cylindracea*	9	11	0	8 (R)	1

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, E_{calc}, and E were not determined.

Appendix IV: Publications

Impact of variation of the acyl group on the efficiency and selectivity of the lipase-mediated resolution of 2-phenylalkanols

Foley, Aoife M.; Gavin, Declan P.; Joniec, Ilona; Maguire, Anita R., *Tetrahedron: Asymmetry*, 2017, *28*, 1144 – 1153.

Hydrolase-mediated resolution of the hemiacetal in 2-chromanols: The impact of remote substitution

Gavin, Declan P.; Foley, Aoife; Moody, Thomas S.; Rao Khandavilli, U. B.; Lawrence, Simon E.; O'Neill, Pat; Maguire, Anita R., *Tetrahedron: Asymmetry*, 2017, *28*, 577 – 585.

Dynamic kinetic resolution of 2-methyl-2-nitrocyclohexanol: Combining the intramolecular nitroaldol (Henry) reaction & lipase-catalysed resolution

Foley, A. M.; Gavin, D. P.; Deasy, R. E.; Milner, S. E.; Moody, T. S.; Eccles, K. S.; Lawrence, S. E.; Maguire, A. R., *Tetrahedron*, 2018, *74*, 1435 – 1443.

Manuscripts in preparation, not included in this appendix:

Patent pending: "A new functional ω-transaminase enzyme" European patent application number: 18186426.5

Isolation and characterization of a novel remote-stereospecific marine transaminase with a unique challenging substrate profile

Abreu-Castilla, I.; Gavin, D. P.; Reen, F. J.; Foley, A. M.; Rocha-Martin, J.; Maguire, A. R.; O'Gara, F., *Manuscript in preparation.*

Identification of a novel esterase isolated from a marine environment which displays an unusual substrate scope and its characterisation as an enantioselective biocatalyst

Declan P. Gavin, Edel J. Murphy, Aoife M. Foley, Ignacio Abreu-Castilla, F. Jerry Reen, Stuart G. Collins, Fergal O'Gara, Anita R. Maguire, *Manuscript in preparation*.